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BULLETIN No. 6

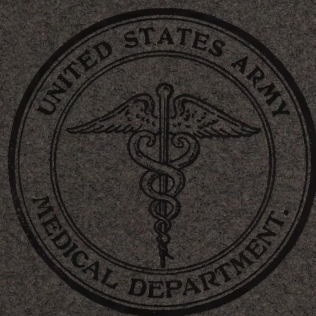
AUGUST, 1914

**THE PROPHYLAXIS OF MALARIA
WITH SPECIAL REFERENCE TO
THE MILITARY SERVICE**

BY

CHARLES F. CRAIG

Captain, Medical Corps, U. S. Army



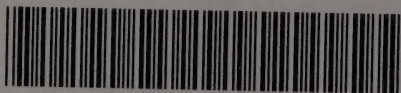
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- No. 1. Photomicrographs of Spirochetæ, Entamebæ, Plasmodia, Trypanosomes, Leishmania, Negri Bodies and Parasitic Helminths. January, 1913.
- No. 2. Papers by Officers of the Medical Corps, U. S. Army, read before the Fifteenth International Congress of Hygiene and Demography. January, 1913.
- No. 3. Studies of Syphilis, by Charles F. Craig, Captain, Medical Corps, U. S. Army, and Henry J. Nichols, Captain, Medical Corps, U. S. Army, with Introduction by Major Frederick F. Russell, Medical Corps, U. S. Army. June, 1913.
- No. 4. Disease-Bearing Mosquitoes of North and Central America, the West Indies, and the Philippine Islands, by C. S. Ludlow, Ph. D., Anatomist, Army Medical Museum. November, 1913.
- No. 5. Mental Disease and Defect in the U. S. Troops, by Captain Edgar King, Medical Corps, United States Army. March, 1914.



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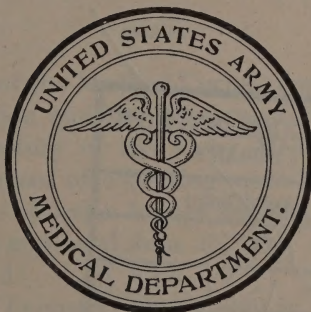
**THE PROPHYLAXIS OF MALARIA
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BY

CHARLES F. CRAIG

Captain, Medical Corps, U. S. Army

PUBLISHED BY AUTHORITY OF THE ACT OF CON-
GRESS APPROVED AUGUST 1, 1914, AND WITH
THE APPROVAL OF THE SECRETARY OF WAR,
FOR THE INFORMATION OF MEDICAL OFFICERS



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PREFACE.

In the sundry civil act for 1914, under the appropriation for printing and binding for the War Department, it was provided:

That the sum of \$3,000, or so much thereof as may be necessary, may be used for the publication from time to time of bulletins prepared under the direction of the Surgeon General of the Army, for the information of medical officers, when approved by the Secretary of War.

Similar provision was made in the sundry civil act for 1915, and it is hoped that this appropriation will be continued from year to year in the future. It is intended that these bulletins shall be used for the publication to the Medical Corps of the special technical work of the service laboratories, the reports of the boards for the study of tropical diseases, and other work of medical officers which is of too special or technical a character to make it acceptable for publication in the medical journals and the Military Surgeon.

The bulletin will be published, if possible, at least quarterly. Officers of the corps are requested to submit suitable articles to this office for use in the bulletin, with the understanding that they may be published elsewhere by the author if found not suitable for the bulletin.

WILLIAM C. GORGAS,
Surgeon General, United States Army.

SEPTEMBER 24, 1913.

ORDER.

A board of officers of the Medical Corps, representing the Sanitary and Statistical Division and the Library of the Surgeon General's Office and the Laboratory of the Army Medical School, is hereby convened for the purpose of collecting materials and arranging for the publication of the bulletins for the instruction of medical officers authorized by the act approved June 23, 1913.

Detail for the board:

Lieut. Col. Champe C. McCulloch, jr.

Maj. William J. L. Lyster.

Maj. Eugene R. Whitmore.

By order of the Surgeon General:

CHAS. M. GANDY,
Lieutenant Colonel, Medical Corps, United States Army.

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THE PROPHYLAXIS OF MALARIA.

INTRODUCTORY REMARKS.

The discovery by Laveran of the parasites concerned in the etiology of the malarial fevers, and by Ross of the method of transmission of these parasites from man to man by the mosquito, has placed the prophylaxis of these infections upon a firm scientific basis; while the discovery of cinchona and its alkaloid, quinine, placed in our hands a true specific against the parasites. In many countries these discoveries have been taken advantage of in extensive campaigns against malaria, and such campaigns have been successful in proportion to the thoroughness with which measures based upon etiological facts have been applied in prophylaxis.

In this bulletin, prepared at the request of the editorial board appointed by the Surgeon General of the Army, special stress will be laid upon those measures that are most applicable in the prophylaxis of malaria in the military service, including service in the field as well as in semipermanent camps and permanent posts. It is obvious that methods of prophylaxis that would be suitable in a permanent post might be impracticable upon the march, in bivouac, and in camps lasting only a day or two, so that it will be necessary to consider somewhat *in extenso* the prophylactic measures suitable under the special conditions brought about by military operations as well as those suited to preventing infection in permanent posts.

At the present time our knowledge of the etiology of malarial infections is so extensive and accurate that no matter how badly a region may be infected or how difficult the local conditions may make the application of prophylactic measures some method may be adopted that will result in success. It is now proven beyond controversy that the malarial fevers are transmitted from man to man by mosquitoes belonging to the *Anophelinae*; that these mosquitoes in order to become infective must have bitten an infected individual; that the malaria parasites undergo a definite cycle of development in both man and the mosquito, which, if interrupted at any stage, will result in the death of the parasites; and that these parasites, so far as we know, exist only in man and mosquitoes, so that it is unnecessary to consider other animal or insect hosts in the prophylaxis of malaria. Therefore it follows that if it were possible to destroy all malaria-carrying mosquitoes or kill all the plasmodia in the blood of all infected individuals we would succeed in the

eradication of these fevers in every locality. Unfortunately, we must admit that in practice we can only hope for a partial success if we depend upon one method of prophylaxis alone, and it is generally necessary to combine methods looking to the destruction of mosquitoes, the protection of man from the bites of these insects, and the destruction of the malaria plasmodia.

That methods based upon the discoveries of Laveran and Ross, when properly applied, are successful has been proven by numerous sanitarians. The success achieved by Gen. Gorgas and Col. Kean, of the Medical Corps, in Habana; of Gen. Gorgas in the Canal Zone; of Celli in Italy; of Watson in the Federated Malay States; and of the Sergeants in Algeria in the prevention of malaria has demonstrated for all time the wonderful value of prophylactic measures against these infections, and there is no more striking illustration of what may be expected from intelligent prophylaxis than is shown in Chart No. I, giving the malarial rates in the Canal Zone since 1906 to 1913. As will be noted, during 1906 nearly 7 per cent of the entire working force on the canal entered hospital each month suffering from malarial infections, while in 1912 less than 1 per cent per month entered hospital from these infections.

In the Army there has been a gradual and continued reduction in the number of cases of malaria since 1898, as shown by Chart No. II. It should be understood, however, that the enormous number of admissions from malarial fevers in 1898, 1899, and even in 1900, as shown in this chart, can not be taken as representing the actual facts, for it is well known that a very large percentage of the cases of typhoid fever occurring during these years, and especially during 1898 and 1899, were diagnosed as remittent malarial fever and so entered upon the records. However, if we accept the year 1901 as representing accurate statistics of this disease, it will be noted that the admission rate for all American troops, serving both at home and in our tropical possessions, has been reduced from 365.39 per 1,000, the rate for 1901, to 24.75 per 1,000, the rate for 1913.

The noneffective rate from malarial infections, the best index we possess of the influence of the disease upon the efficiency of the Army, has likewise fallen greatly. This rate for all American troops, as shown in Chart III, serving both at home and abroad, was 4.46 in 1903, while in 1913 it was only 0.53, the lowest noneffective rate from these fevers since 1898.

In the troops serving in the United States the reduction in malaria, as would be expected, has been more marked than in the Philippines. In Chart No. IV is given the admission and noneffective rates for these fevers since 1904, and it will be noted that there has been a steady decrease in both rates, until in 1913 we

CHART No. 1.

MALARIA IN THE CANAL ZONE, ISTHMIUS OF PANAMA

Malaria Cases among the employees of the Isthmian Canal Commission

FOR THE YEARS 1906-1907-1908-1909-1910-1911-1912

This chart shows the number of Malaria cases sent to Hospitals each month, expressed as a percentage of the entire working force.

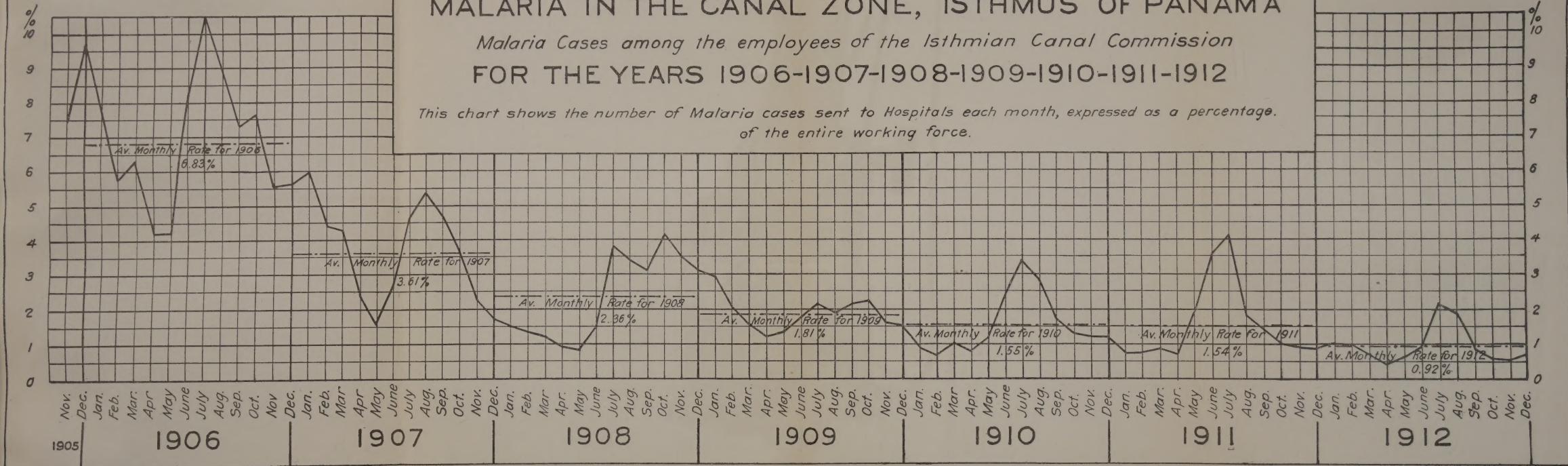


CHART NO. II.

Malarial Fevers.
Admission Rates.
Admission rates for Malarial Fevers, the
Army, American troops {enlisted men} years, 1898-1913.

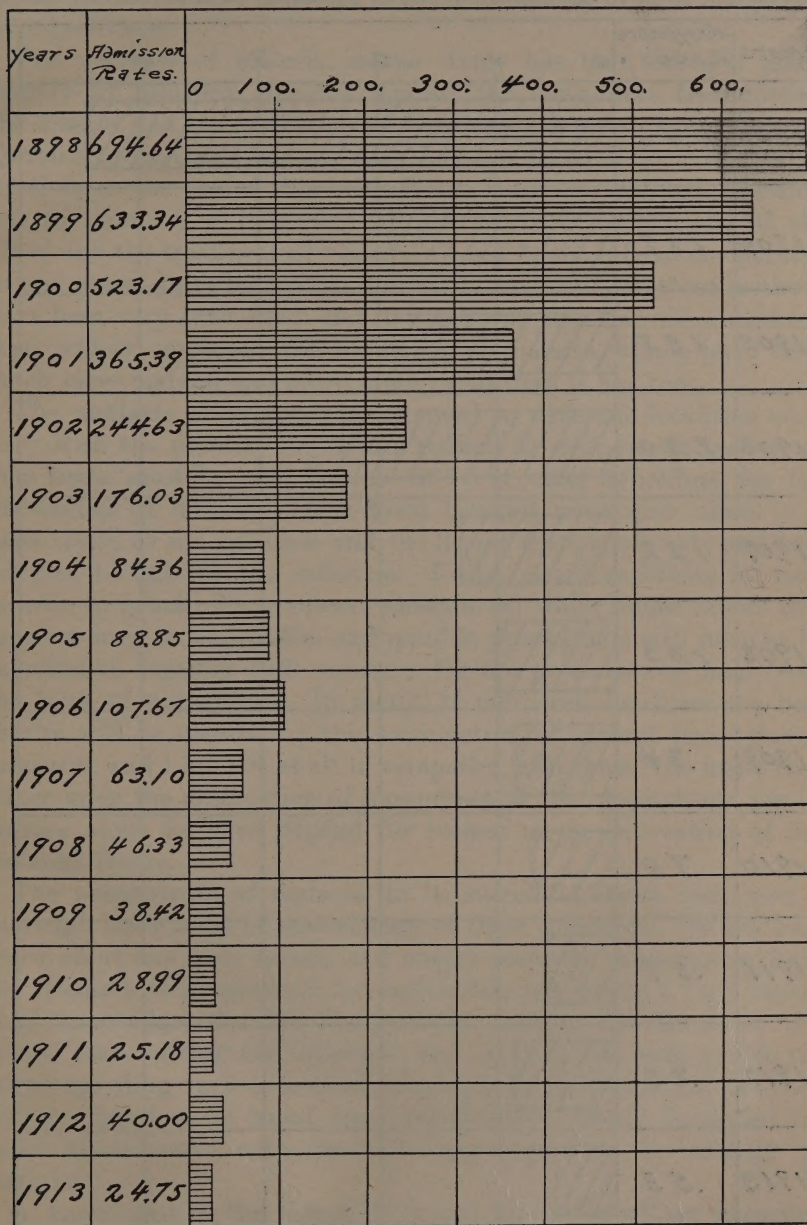
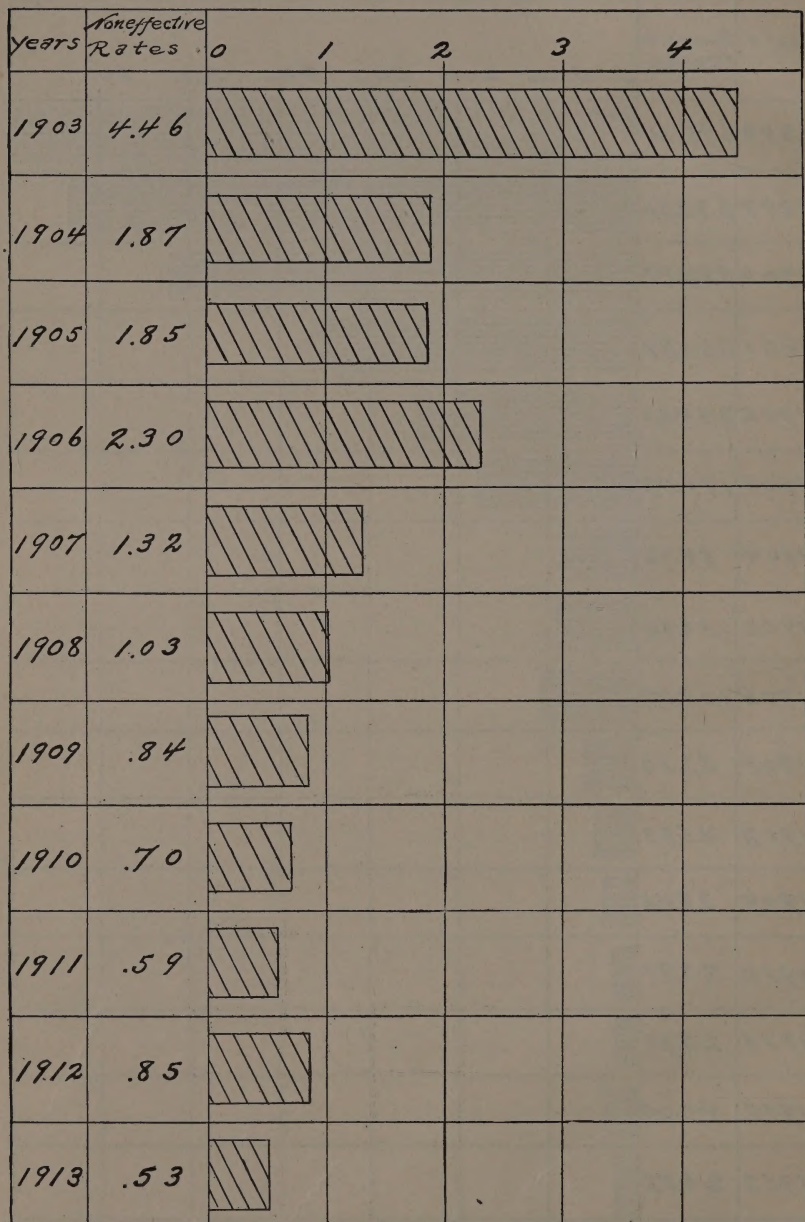


CHART No. III.

*Malarial Fevers.
Noneffective Rates.*

*Noneffective rates for Malarial Fevers, the
Army, American troops {enlisted men} years, 1903-1913.*



reached the very small noneffective rate of 0.18 and the admission rate of 8.79. In the Philippines on the other hand (Chart No. V) the decrease has not been as steady, and in 1912 and 1913 the admission rates are higher than in 1910 or 1911. The exact reason for this increase during the years mentioned is not known but it was probably due to more exposure in the field because of local maneuvers or expeditions.

The decrease of malaria in the Army has been brought about largely by measures directed against the mosquitoes transmitting the disease, and the protection of man from the bites of these insects. Quinine prophylaxis has probably had but little to do with this reduction, as the use of this method has been very limited. Neither has the control of treatment by microscopic examinations of the blood and the treatment of "carriers" and latent infections operated to any extent in reducing the disease in the Army, as these methods have been very little used, and it is believed that had these methods been widely employed the reduction of malaria would have been much more marked and much more rapid than it has been.

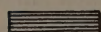
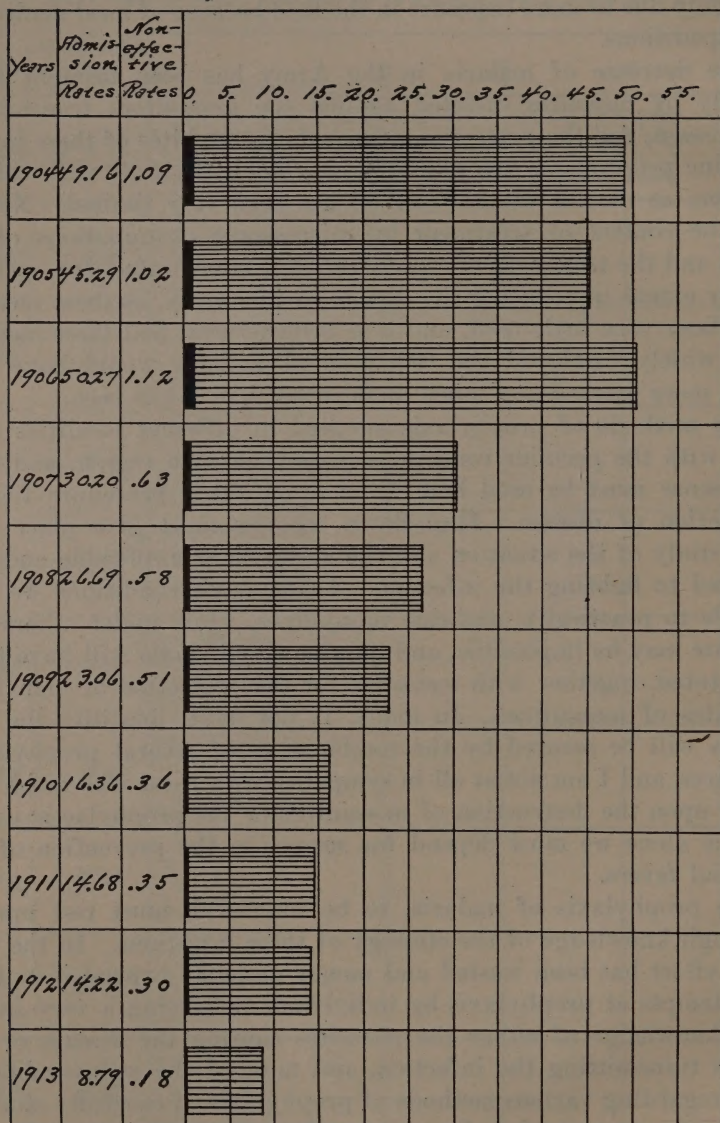
The methods of prophylaxis adopted in different localities must vary with the peculiar conditions present in each region, and common sense must be used here as in every other procedure for the prevention of disease. High-flown theories must give place to a calm study of the situation and the means most applicable and best adapted to fighting the infection. Under some conditions we may be able to practically eradicate mosquitoes, while under others this measure may be impossible, and quinine prophylaxis will have to be substituted, together with measures for the protection of man from the bites of mosquitoes. In many, if not most, localities the best results will be secured by the combination of several prophylactic measures, and I am not at all in sympathy with those who insist that either upon the destruction of mosquitoes or the prophylactic use of quinine alone we must depend for success in the prevention of the malarial fevers.

The prophylaxis of malaria, to be successful, must rest upon a thorough knowledge of the etiology of these infections. In the past much effort has been wasted and money uselessly expended in foolish attempts at prophylaxis by individuals possessing a very superficial knowledge of either the parasites causing the disease or the insects transmitting the infection, and most of the unfavorable reports regarding various methods of prophylaxis, if carefully studied, will be found to be based upon insufficient evidence furnished by those unacquainted with the real facts regarding the etiology of malaria.

A knowledge of the morphology and life cycle of the parasites causing malaria and of the mosquitoes transmitting the disease is

CHART No. IV.

Malarial Fevers.
Admission and Noneffective Rates.
Admission and Noneffective rates for Malarial
Fevers for the United States, American Troops,
enlisted men } years, 1904-1913.

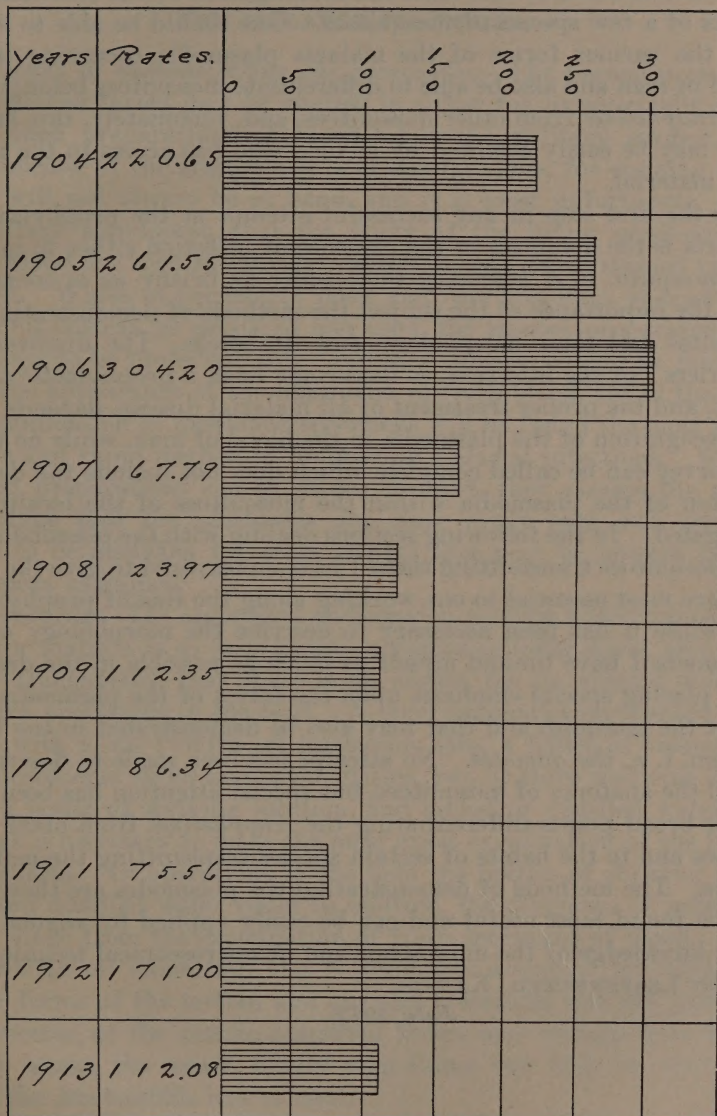


Admission rates.

Noneffective rates



CHART No. V.

Malarial Fevers.
*Admission Rates.**Admission rates for Malarial Fevers for the
Philippine Islands, American Troops {enlisted men}
years, 1904-1913.*

absolutely essential to one who hopes to achieve success in prophylaxis. This does not mean that one must be a professional protozoologist or entomologist, for a good working knowledge of these subjects may be obtained from the careful study of the blood from a few cases of malaria and from the investigation of the structure and habits of a few species of mosquitoes. One should be able to recognize the various forms of the malaria plasmodia occurring in the blood of man and also be able to differentiate mosquitoes belonging to the *Anopheles* from other mosquitoes, and, fortunately, this knowledge may be easily acquired by anyone who has access to the necessary material.

As the first step in any successful attempt at the prophylaxis of malaria is the diagnosis of the presence of infection either in man or the mosquito, it is necessary to consider as briefly as is consistent with the importance of the subject the methods of demonstrating the parasites and their morphology and life cycle. The discovery of "carriers" of the infection, so important from a prophylactic standpoint, and the proper treatment of all malarial disease, depends upon the recognition of the plasmodia in the blood of man, while no malaria survey can be called complete which does not include the demonstration of the plasmodia within the mosquitoes of the locality investigated. In the following sections dealing with the plasmodia and the mosquitoes transmitting them I have endeavored to give the facts that are most essential to one working along the line of prophylaxis; and while it has been necessary to describe the morphology of the plasmodia I have limited myself as much as possible in the description, placing special emphasis upon the forms of the plasmodia that infect the mosquito and that may also be demonstrated in the blood of man, i. e., the *gametes*. No attempt has been made to describe in detail the anatomy of mosquitoes, but special attention has been paid to the broad points differentiating the *Anopheles* from other mosquitoes and to the habits of certain species transmitting the malarial fevers. The methods of demonstrating the plasmodia are those that I have found most useful and can be easily applied by anyone having a knowledge of the microscope and of microscopical technique.

FORT LEAVENWORTH, KANSAS,

July, 1914.

CHAPTER I.

THE MALARIA PLASMODIA.

Methods of demonstrating the plasmodia.—The parasites causing the malarial fevers may be studied in either the living condition or in stained preparations of blood. For the military surgeon the examination of the fresh blood is of special value, for staining solutions will not always be at hand, and it is most unfortunate, from a military standpoint, that the study of the living plasmodia in fresh blood has been so entirely replaced by the use of stained preparations. While, at permanent posts and base hospitals, where staining solutions can be prepared and used, the stained preparations of blood are to be preferred, every military surgeon should be familiar with the appearance of the plasmodia in fresh blood, for in the field the examination of unstained specimens will be found the most practicable and rapid method of diagnosing malarial infections. Where stained preparations can be made, however, they possess the great advantage that the plasmodia are more easily seen and are less liable to be mistaken for other objects that may be present in the specimens examined.

Apparatus.—The apparatus necessary in making a blood examination for malaria is so very simple, occupies so little space, and is so easily transported, that there is no excuse for its absence even in military operations in the field. A good compound microscope provided with a one-twelfth oil immersion lens, a bottle of immersion oil, microscopic slides and cover-glasses, two or three medicine droppers, a bottle of staining solution, and boiled or distilled water are all that is necessary to make the most exact examination of the blood for malaria. In operations in the field the bottles of staining solution and distilled water could be omitted, the diagnosis of infection being made from the examination of fresh specimens of blood. The one-twelfth oil immersion lens is absolutely essential, for while the larger forms of the tertian and quartan plasmodia and the *gametes*, or *crescents*, of the aestivo-autumnal fevers may be seen with lower power lenses, the small, hyalin ring-forms can only be well seen when the one-twelfth lens is used.

Preparation of living specimens.—The following procedure is recommended if it is desired to procure *fresh* specimens of blood for examination for the plasmodia. The patient's ear lobe or the tip of the finger is carefully cleaned with alcohol, dried thoroughly, a

slight puncture made with a lancet or needle, and the first drop or two of blood allowed to flow away. A very small drop is then taken upon the center of a microscopic slide which has been carefully cleaned and a cover glass placed gently over it. If the slide is clean and the drop of blood small the weight of the cover glass will cause the blood to spread evenly and quickly beneath it; but if it does not, very slight pressure will suffice to spread the blood and will do no harm. If upon examination it is found that the blood cells are in clumps or rouleaux, the preparation should be discarded and one prepared in which the red cells are spread singly and evenly over the microscopic field. The preparations should be examined as soon as possible; but if carefully wrapped in tissue paper, they may be carried in the pocket for several hours without much danger of changes occurring which will obscure the plasmodia; and if the specimens be carefully ringed with vaseline, they will keep for as long as 12 hours. The ear is preferable to the finger for obtaining blood, especially in children, as there is less pain and the patient can not watch the operator during the procedure. At least a half hour should be spent upon the examination of the specimen before a negative report is returned, and at least three or four preparations should be examined. In the vast majority of active infections, however, a few moments examination will disclose the plasmodia, but where a search is being made for latent infections and "carriers" it is not safe to consider a case as negative unless several specimens are carefully examined.

Preparation of stained specimens.—All that is necessary in the way of apparatus for preparing blood smears for staining are microscopic slides and a needle with which to make the puncture in the ear-lobe or finger. After cleansing the ear or finger, as described, a puncture is made with the needle and a small drop of blood caught upon the surface of a clean microscopic slide, near one end; as quickly as possible the end of another slide is placed in contact with the drop of blood, either before or behind it, and the blood allowed to spread along the edge of the applied end. As soon as this occurs the upper or applied slide is pushed or drawn gently along the surface of the slide containing the drop of blood, and when this is properly done a thin even smear is obtained. Several smears should be prepared from each individual examined.

Method of staining.—Many methods have been devised for staining the malaria plasmodia, but I have found Wright's modification of the Romanowsky stain as satisfactory as any, and it is absolutely reliable when the staining solution is properly prepared and used. Considerable time and care are necessary in the preparation of the stain, and this has been urged as an objection to its use; but anyone who will carefully follow the directions that follow will have no

trouble in securing a reliable stain, and, in time of active field operations, if it were desired to issue the powder, it could be prepared in large amounts at the various department laboratories and issued in sealed glass tubes, each tube containing enough powder for a definite amount of staining solution, and with the powder could be sent enough of the proper methylic alcohol to make up the amount of solution indicated.

The powder used in preparing the staining solution is obtained as follows: In a flask add 0.5 gram of c. p. sodium bicarbonate to 100 c. c. of distilled water; dissolve thoroughly, and slowly add, while shaking, 1 gram of Grubler's methylene blue; heat for one hour in an Arnold sterilizer after the steam is up, and then cool the solution. A considerable amount of the methylene blue will remain undissolved, but this should be allowed to remain in the solution.

Make a solution of Grubler's yellow aqueous eosin by adding 1 gram of eosin to 1,000 grams of distilled water. Add this slowly, while stirring, to the cooled methylene blue solution which has been poured into a white dish or bowl. The eosin solution is added until a well-marked precipitate appears and the surface of the mixture is covered with a greenish metallic scum. Test repeatedly, while adding the eosin solution, by placing a drop of the mixture upon a piece of white filter paper. When sufficient of the eosin solution has been added a well-marked pink halo should surround the small amount of blue precipitate left upon the paper. Now allow the mixture to stand for 15 minutes and then filter through one small filter paper; the precipitate is saved, dried in a hot-air oven at 60° C., the greenish mass thus obtained powdered, and stored in an air-tight bottle. Large amounts of the powder may be prepared by increasing the amounts of the reagents given, in proper ratio.

The staining solution.—The solution used in staining the blood smears is prepared by taking 0.3 gram of the powder and adding it to 100 c. c. of pure methylic alcohol (this *must* be Merck's reagent alcohol) filter, and add enough alcohol to bring the entire amount to the original 100 c. c. Let stand for an hour or two before using.

Method of use.—Add a few drops of the staining solution to the blood smear and let stand for from three to five minutes. This fixes the specimen. Then add enough distilled water to cause a slight greenish metallic scum to form upon the surface of the solution; let stand for five minutes, wash in running distilled water, and examine when dry. The exact time for staining, after the addition of the distilled water, varies somewhat with different specimens of blood, but a little experience will soon enable one to judge the right time.

The final washing with distilled water is very important, as by it the precipitate formed during the staining process is removed and the differentiation of the staining of the cytoplasm of the erythrocyte

and the chromatin of the plasmodia is obtained. The washing should be continued until the specimen becomes a delicate shade of pink or pinkish-brown. If distilled water can not be obtained, thoroughly boiled water may be used instead.

Staining reactions with Wright's stain.—In specimens of blood properly stained with Wright's stain, the red blood corpuscles are stained a brownish-pink or light salmon, while the various leucocytes are stained as follows: The polynuclear leucocytes present a violet nucleus with unstained cytoplasm except for light pink granules; the mononuclears and lymphocytes have a dark ruby-red or violet nucleus, while the cytoplasm is stained blue; the eosinophiles have a violet or bluish-violet nucleus, while the granules are stained red; the mast cells have purplish-black granules and a dark violet nucleus; while the blood plates stain a ruby-red color, with, in most instances, a more or less definite pale lilac margin.

The cytoplasm of the malaria plasmodia stains a robin's egg blue, the vesicular portion of the nucleus remains unstained, while the chromatin of the nucleus stains a ruby-red or brilliant violet color. The pigment generally appears greenish in color in the tertian plasmodia and almost black in the quartan and estivo-autumnal plasmodia.

The Ross thick-film method.—In suspected cases of malaria in which the plasmodia can not be demonstrated by the methods described, the thick-film method of Ross should be tried. This method will be found of special service in the search for "carriers" of the infection and should always be employed in a malaria survey before a locality is returned as free from the disease.

A large drop of blood is placed upon a microscopic slide at or near the middle, and is spread with a needle until it covers an area about one-half inch in diameter. The smearing may be done with a platinum loop, and the film should be made as evenly as possible. The preparation is now placed aside and allowed to dry.

After the film is dry, the slide is placed in a mixture composed of 50 c. c. of commercial ethyl alcohol containing 10 drops of commercial hydrochloric acid, and removed when the hemoglobin is completely dissolved—a process which is usually complete in from 10 minutes to half an hour, varying with the thickness of the blood smear. The preparation is now fixed and should be washed in running water for 15 minutes in order to remove the acid, which entirely prevents staining if any considerable amount remains upon the slide. After washing, the specimen is allowed to dry and stained with Wright's stain in the manner already described. More stain should be used in covering the film, and a longer time should be allowed for staining because of the thickness of the film of blood.

After staining, the preparation is washed in running tap water until no more blue color comes from the film. The specimen is then allowed to dry, after which it is ready for examination.

In specimens stained in this manner it should be remembered that, owing to the dissolving of the hemoglobin by the acid, the cytoplasm of the red blood corpuscles is not distinctly stained, the intracellular plasmodia appearing to be free in the plasma. The entire film is more or less pink in color, the plasmodia appearing as blue bodies containing more or less red chromatin against the pinkish background. The tertian and quartan plasmodia, if beyond the ring stage in growth, appear as irregular blue masses containing granules of red chromatin, while the ring forms appear as blue rings containing one or two red chromatin granules, or as minute, irregular bodies, if viewed at an angle. The *gametes* are easily distinguished, although the crescentic *gamete* of the estivo-autumnal plasmodia often appears distorted. Some experience is required to identify the plasmodia in these thick films, but a little practice will soon enable one to become expert in the use of this very valuable method of demonstrating the malaria plasmodia.

THE MORPHOLOGY AND LIFE CYCLE OF THE MALARIA PLASMODIA.

The malaria plasmodia were first described by Laveran³ in 1880 and are found in man upon and within the red blood corpuscles. In this situation they feed upon and destroy the corpuscles, producing thus the anaemia always present in every malarial infection, and by their sporulation the symptom complex which we know as the malarial paroxysm. Although the *classification* of these parasites has occupied the attention of zoologists for years, it can not be said that there is yet a complete agreement regarding the exact position of the species causing malaria in man. All are agreed that they belong to the Protozoa, and most authorities place them in the *Sporozoa*, and in the order *Hæmosporidia*. At least three species are recognized by the vast majority of observers—i. e., *Plasmodium vivax* (the tertian parasite), *Plasmodium malariae* (the quartan parasite), and *Plasmodium falciparum* (the estivo-autumnal parasite). The latter species has been divided by many observers into two varieties, the tertian estivo-autumnal plasmodium and the quotidian estivo-autumnal plasmodium. The tertian species should be called *Plasmodium falciparum*, while I⁴ have proposed the name *Plasmodium falciparum quotidianum* for the parasite causing the quotidian type of estivo-autumnal malaria, believing it to be a subspecies of *Plasmodium falciparum*.

In describing the malaria plasmodia two distinct life cycles must be considered—first, the human or asexual cycle, called *schizogony*, occurring in man; and second, the mosquito or sexual cycle, called *sporogony*, occurring in the infected mosquito. In each of these cycles of development the morphology of the parasites varies greatly and should be familiar to everyone engaged in the prophylaxis of the malarial fevers.

In the following descriptions the morphology of the various species of human plasmodia will be first given as observed in living preparations and then as observed in stained preparations. The forms pertaining to the human cycle of development will first be described and then the forms concerned in the mosquito cycle.

Morphology of Plasmodium vivax (the tertian parasite) (Schizogony or human cycle).—As is well known this species of malaria plasmodium completes its cycle of development in the blood of man in 48 hours and produces the well-known type of malarial fever associated with a chill and fever occurring every other day.

The parasite is first observed, in *unstained* preparations, within or upon the red blood corpuscle as a small, nonmotile, hyaline ring or disk, the *trophozoite*, measuring about 2 microns in diameter; its outline is very indistinct, and it is often overlooked owing to lack of amoeboid movement and the delicate veil-like consistency of its cytoplasm. As it grows older, becoming the *schizont*, it develops marked amoeboid movement, but is still indistinct in outline until pigment is developed at the end of from six to eight hours. The pigment is reddish brown in color and arranged irregularly throughout the cytoplasm in the form of very fine granules. As development proceeds the pigment becomes motile, due apparently to currents within the cytoplasm of the parasite, while the amoeboid motion of the plasmodium becomes less pronounced. As growth proceeds the pigment increases gradually in amount and remains active until just before sporulation when it becomes collected in large masses or a single large irregular mass near the center of the parasite.

At the end of 24 hours the plasmodium fills more than half of the infected red corpuscle, contains much actively motile pigment, and varies greatly in shape due to the marked amoeboid activity of the organism. The infected red cell is considerably larger than normal and lighter green in color. At the end of 36 hours the plasmodium has attained its greatest size and practically fills the infected corpuscle. Amoeboid motion is sluggish, but the pigment, which has still further increased in amount, is very actively motile and is distributed in the form of fine granules throughout the cytoplasm. The cytoplasm of the plasmodium is colorless, but the organism is sharply outlined. The infected red corpuscle is almost twice the size of the normal corpuscles surrounding it. At the end of 48 hours sporula-

tion occurs; the pigment becomes collected near the center or to one side of the plasmodium in the form of a compact clump or clumps, and fine radial striations are observed extending from the center toward the periphery of the plasmodium, dividing it into several ovoid segments or spores. As a rule, these spores, in the tertian plasmodium, are arranged in two rows, one row surrounding the center of the plasmodium and another surrounding the first row; but often the spores are arranged irregularly and are always devoid of pigment. They vary in number from 12 to 24, or even more, but the average is from 16 to 20. The spores are known as *merozoites* and when free in the blood plasma measure from one-fifth to 2 microns in diameter, are oval in shape, hyaline in appearance, and present a spherical, refractive center, and a less refractive mass of protoplasm surround it. The *merozoites* are capable of infecting the red blood corpuscles and thus the human life cycle of the plasmodium is continued.

EXPLANATION OF PLATE 1.

- FIGURE 1.—*Plasmodium vivax*. (Tertian plasmodium.) Young forms or the so-called "ring forms." Wright's stain. $\times 1200$.
 FIGURE 2.—*Plasmodium vivax*. Quarter-grown parasite. Wright's stain. $\times 1200$.
 FIGURE 3.—*Plasmodium vivax*. Half-grown parasite. Wright's stain. $\times 1500$.
 FIGURE 4.—*Plasmodium vivax*. Three-quarters-grown parasite. Wright's stain. $\times 1800$.

EXPLANATION OF PLATE 2.

- FIGURE 1.—*Plasmodium vivax*. (Tertian plasmodium.) Pre-sporulating parasite. Wright's stain. $\times 1800$.
 FIGURE 2.—*Plasmodium vivax*. Sporulating parasite. Wright's stain. $\times 1200$.
 FIGURE 3.—*Plasmodium vivax*. Sporulating parasite. Wright's stain. $\times 1800$.
 FIGURE 4.—*Plasmodium vivax*. Sporulating parasite. Wright's stain. $\times 1800$.

EXPLANATION OF PLATE 3.

- FIGURE 1.—*Plasmodium vivax*. (Tertian plasmodium.) Free spores or *merozoites*. Wright's stain. $\times 1200$.
 FIGURE 2.—*Plasmodium vivax*. Fully developed *macrogametocyte* of *Plasmodium vivax*. $\times 1800$.
 FIGURE 3.—*Plasmodium vivax*. Fully developed *microgametocyte* of *Plasmodium vivax*. Wright's stain. $\times 1800$.
 FIGURE 4.—*Plasmodium vivax*. Atypical parasite, resembling the *parthenogenetic macrogametes* described by Schaudinn. Wright's stain. $\times 1500$.

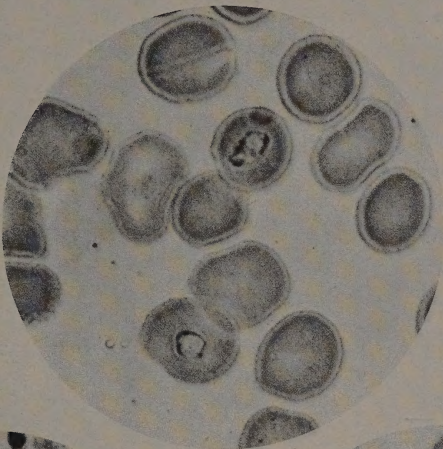
In preparations stained with Wright's stain, the youngest form of *Plasmodium vivax* appears in the red cell as the so-called "ring form" consisting of a delicate ring of cytoplasm stained a robin's-egg blue, at one portion of which is a ruby-red dot of chromatin, the ring inclosing an unstained area through which the salmon or pink of the corpuscle is visible. In those plasmodia in which ameboid

motility was present at the time of fixation many irregular forms of the "ring" are observed, due to minute pseudopodia arising from the periphery of the organism. Delicate, blue-stained filaments of cytoplasm may be observed spreading over the infected erythrocyte and the chromatin mass may be situated at any portion of the parasite, quite often within one of the pseudopodia. As the *schizont* becomes larger the blue-stained cytoplasm is observed to contain greenish pigment and the single dot of chromatin has increased until the cytoplasm contains threads and granules of this substance stained a brilliant red. At a certain period of development the chromatin divides into such fine filaments and grains that in the stained preparations the parasites at this stage appear to be almost devoid of this substance but prolonged staining will generally demonstrate a collection of very fine chromatin granules inclosed within an unstained area, the vesicular portion of the nucleus. As sporulation approaches the cytoplasm stains more intensely blue and the chromatin becomes arranged in irregular clumps throughout the cytoplasm. The sporulating plasmodia present a cytoplasm filled with spherical or oval masses of red chromatin and careful examination will demonstrate that each chromatin mass is surrounded by a blue-stained ring of the cytoplasm; the pigment is collected into one or more irregular masses within the organism along with some residual cytoplasm which stains a pale blue. Not infrequently free spores, or *merozoites*, are observed in stained preparations, and are composed of a ring-shaped or almost solid mass of blue-stained cytoplasm containing a small bright red dot of chromatin.

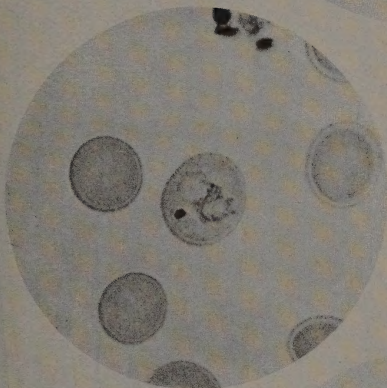
Morphology of Plasmodium malariae (the quartan plasmodium) (Schizogony or Human Cycle).—This species of malaria plasmodium completes its development in man in 72 hours, producing that type of the disease characterized by a chill and a rise in temperature at the end of every third day. With the exception of the quotidian form of estivo-autumnal infection, this is the most uncommon form of malaria.

Like the tertian plasmodium, the organism causing quartan malarial infections appears at first, in the *living* specimen, as a small actively ameboid hyaline body within or upon the red blood corpuscle. It will be noticed that ameboid motion is less marked than in the tertian parasite, and that the youngest forms are smaller than the youngest forms of the latter species. The quartan parasite rapidly becomes pigmented, the pigment being dark brown in color, less motile than the pigment of the tertian plasmodium, and arranged around the periphery of the organism. The outline of the parasite, at every stage of development, is much more distinct than is that of the tertian parasite, and the infected red corpuscle, instead

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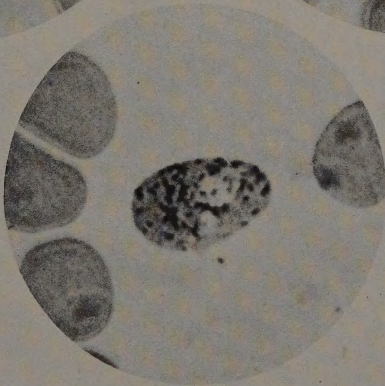
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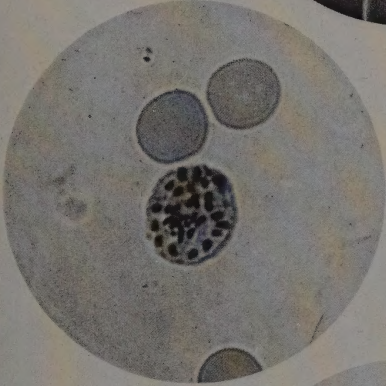
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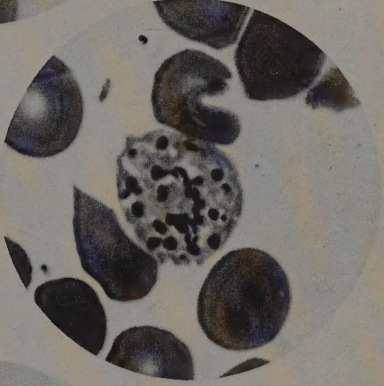
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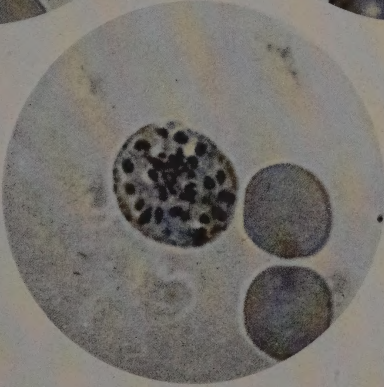
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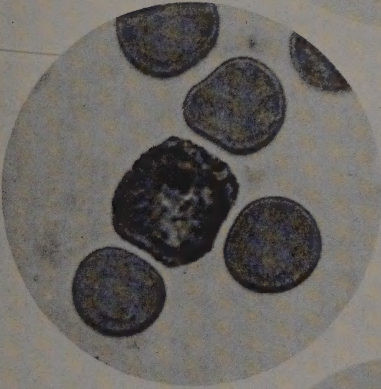
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The *merozoites* or spores consist of a deep blue stained mass of cytoplasm containing a compact clump of dark red chromatin situated somewhere near the periphery. In most instances the mass of chromatin is surrounded by a clear, unstained halo representing the unstained portion of the nucleus.

Morphology of Plasmodium falciparum. Tertian estivo-autumnal plasmodium (Schizogony or Human Cycle).—As I have stated, I believe that there are two distinct varieties of the estivo-autumnal plasmodium, the tertian and quotidian. These are distinguishable microscopically, and the symptoms produced by them are characteristic and easily differentiated clinically. The most common species is the tertian or *Plasmodium falciparum*, and this will first be described.

EXPLANATION OF PLATE 4.

FIGURE 1.—*Plasmodium malariae*. (Quartan plasmodium.) Young parasites. The so-called ring forms. Wright's stain. $\times 1500$.

FIGURE 2.—*Plasmodium malariae*. Half-grown parasite. The so-called band form. Wright's stain. $\times 1800$.

FIGURE 3.—*Plasmodium malariae*. Three-quarters-grown parasite. Wright's stain. $\times 1500$.

FIGURE 4.—*Plasmodium malariae*. Large band form. Wright's stain. $\times 1800$.

EXPLANATION OF PLATE 5.

FIGURE 1.—*Plasmodium malariae*. (Quartan plasmodium.) Pre-sporulating parasite. Wright's stain. $\times 1500$.

FIGURE 2.—*Plasmodium malariae*. Sporulating parasite. Wright's stain. $\times 1200$.

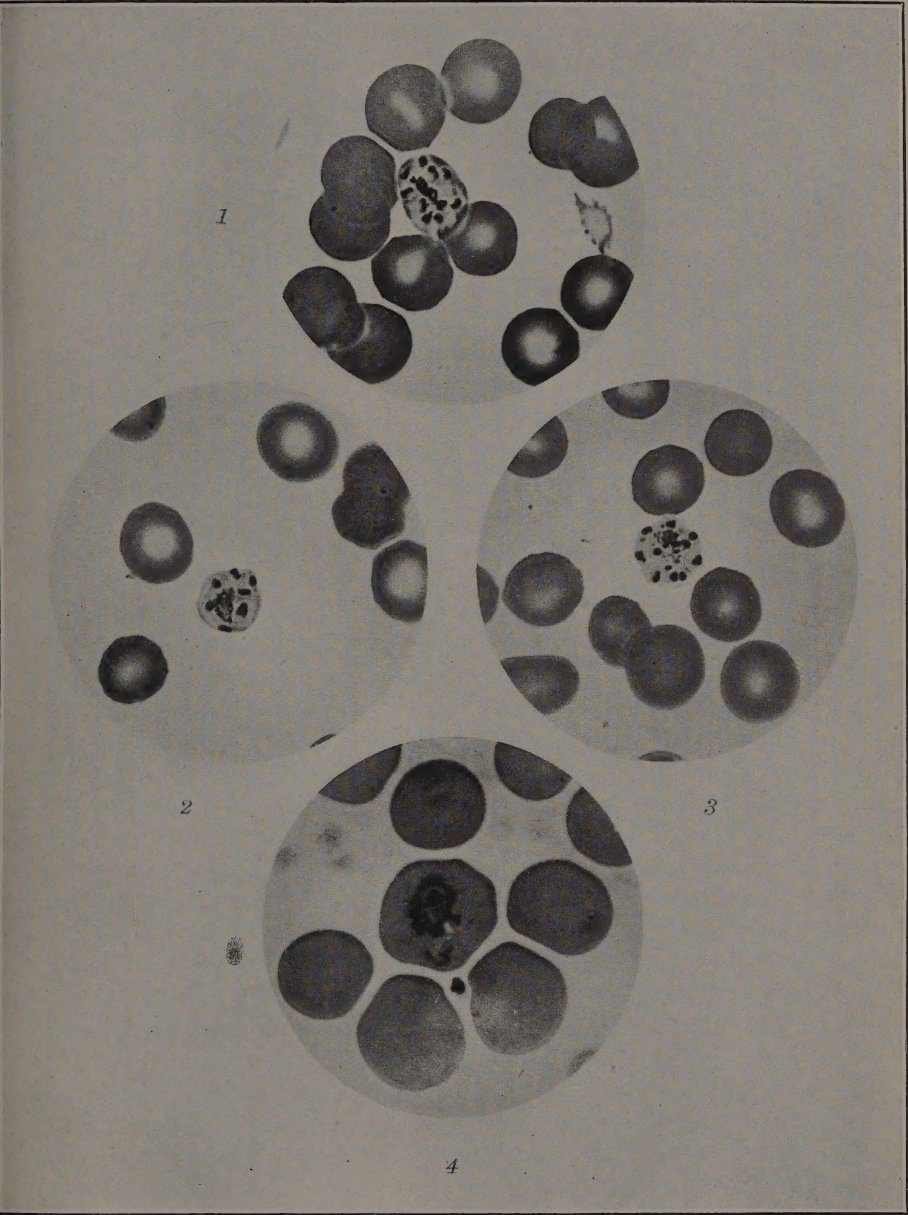
FIGURE 3.—*Plasmodium malariae*. Sporulating parasite. $\times 1200$.

FIGURE 4.—*Plasmodium malariae*. Young gamete. $\times 1800$.

Plasmodium falciparum appears first within or upon the infected red corpuscle as a round hyaline ring or disk. The infected cell is greenish in color, smaller than the normal corpuscles surrounding it, and generally crenated. These young "ring-forms" are irregular in outline, one portion of the ring being larger than the rest, giving it the so-called "signet-ring" appearance. The organism is quite actively ameboid and only rarely is there more than two parasites observed within one corpuscle. In the course of from 20 to 24 hours the hyaline forms become pigmented, the pigment occurring in the form of fine, reddish-brown granules somewhat resembling those found in the tertian parasite. The pigment is in larger amount than in the quotidian plasmodium and is generally motile. As soon as pigmentation occurs the parasites collect in the internal organs, and it is therefore rare to find the half and fully grown forms of this plasmodium in the peripheral blood.

As growth increases ameboid motion becomes lost and at the time of segmentation or sporulation, which occurs at the end of 48 hours





in this species, ameboid motility is entirely absent. At this time the fully developed parasite fills slightly more than half of the infected cell, the pigment is collected at the center in a small compact almost black mass, and radial striations are noted dividing the parasite into from 10 to 15 segments or *merozoites*. In some instances as high as 24 *merozoites* have been observed. Sporulation occurs within the red blood corpuscle and considerable of the infected cell is still undestroyed at the time that sporulation begins. The sporulating forms occur very rarely in the peripheral blood, but are present in large numbers in the capillaries of the internal organs, notably the spleen, liver, and brain, and in the bone marrow.

In *stained* preparations, using the Wright method, the staining reactions are the same as occur in *Plasmodium vivax* and *Plasmodium malariae*, the differential diagnosis resting upon certain morphological features to be noted later in this contribution.

Morphology of Plasmodium falciparum quotidianum. Quotidian estivo-autumnal plasmodium (Schizogony or Human Cycle).—The quotidian estivo-autumnal plasmodium, in the *living* condition, is noticed first within or upon the red blood corpuscle as a very minute ring-shaped or disk-like hyaline body, which upon close inspection is seen to have a very active ameboid motion, the periphery of the parasite continually changing in appearance, due to the protrusion and retraction of minute pseudopodia. The outline of the organism at first is indistinct, but becomes more distinct as growth proceeds, and when the parasite is fully developed the outline is very clear cut and the organism is very refractive. The infected red corpuscle is generally smaller in size than the normal corpuscle, darker green or “brassy” in color, and frequently crenated, and in many instances triple infection of the corpuscle is observed. In the peripheral blood the hyaline, round or “ring-shaped” organisms are those which are most frequently observed, although a small number of the younger pigmented forms are not uncommon. The pigment consists of a small, solid block or mass, almost black in color, situated at some portion of the periphery of the parasite, or at the center, and is never motile. Very rarely the pigment consists of fine granules, but the granules never number more than three or four.

In this species of plasmodium sporulation occurs at the end of 24 hours, and the sporulating forms are very rarely observed in the peripheral blood, although blood from the spleen taken at the proper time will present numerous segmenting organisms. Just before sporulation the plasmodium occupies a little more than one-fourth of the infected corpuscle, thus easily distinguishing it from *Plasmodium vivax* or *Plasmodium malariae*, which entirely fill the red blood cell when fully developed. As sporulation begins the pigment

collects in a small perfectly spherical mass at the center of the parasite, and radial striations may be detected dividing it into from 16 to 18 very minute round or oval segments, or *merozoites*. While in infections with the tertian and quartan plasmodia it is often almost impossible to distinguish the remains of the red corpuscle when sporulation occurs, in this species it can be plainly seen that sporulation occurs before the corpuscle is entirely destroyed, as the sporulating organism occupies less than half of the cytoplasm of the red cell.

In *stained* specimens this species presents the same staining reactions as the other malaria plasmodia, but differentiation is comparatively easy owing to the minute size of this organism.

The morphology of the various malaria parasites so far described is that peculiar to the forms that develop *alone in man*, but in every variety of malarial infection certain forms of the plasmodia are noted that are intended to complete their life cycle within the mosquito, and which, so far as definite evidence now goes, never com-

EXPLANATION OF PLATE 6.

FIGURE 1.—*Plasmodium falciparum*. (Estivo-autumnal plasmodium.) Young parasites. The so-called "ring forms." Wright's stain. $\times 1200$.

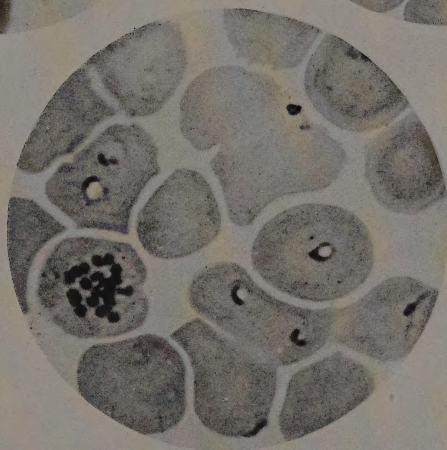
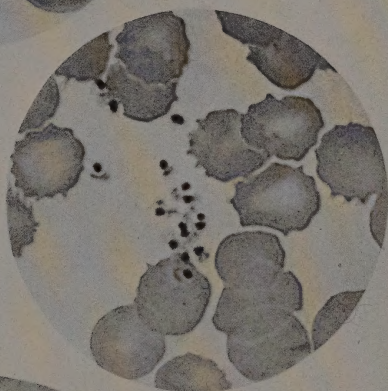
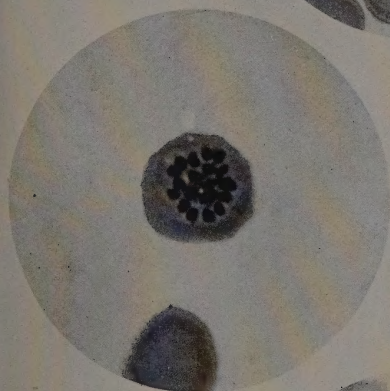
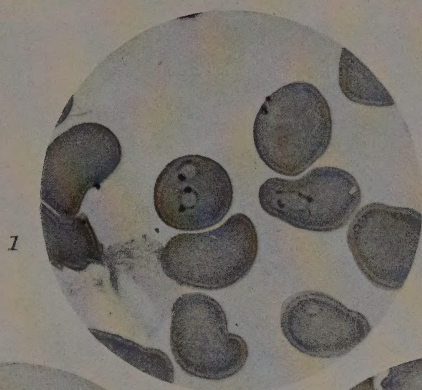
FIGURE 2.—*Plasmodium falciparum*. Sporulating parasite and several young "ring forms." Wright's stain. $\times 1800$.

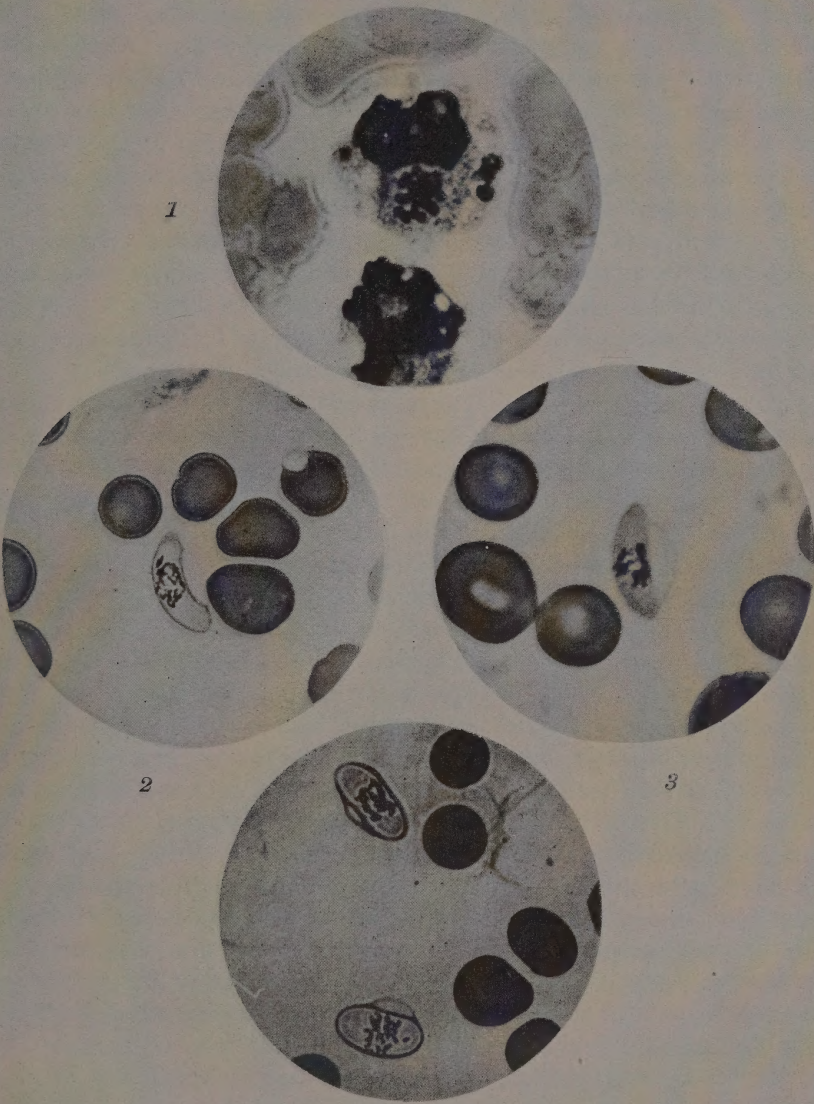
FIGURE 3.—*Plasmodium falciparum*. Sporulating parasite. Wright's stain. $\times 1800$.

FIGURE 4.—*Plasmodium falciparum*. Free spores or *merozoites*. Wright's stain. $\times 1500$.

plete a life cycle within man. These forms are known as *gametes* and probably develop from *sporozoites* in answer to certain influences produced upon the *schizonts* by life within man. While certain authorities believe that the *gametes* are introduced as such by the mosquitoes or develop from certain differentiated *sporozoites*, the great mass of evidence is in favor of their development within man from certain *schizonts*, for it is well known that they do not appear until an infection has lasted for several days, and never appear if the infection is properly treated. These forms will now be described, but before doing so it will be necessary to briefly sketch the life cycle of the malarial plasmodia within the mosquito in order to understand the names applied to the various stages during this cycle of development.

Development of the malaria plasmodia within the mosquito (Sporogony or mosquito cycle).—In tertian and quartan infections certain of the plasmodia are observed that do not sporulate, but remain unchanged in the blood of man until they are removed, when changes occur that normally should occur in the stomach of the





mosquito. These bodies are circular in shape when fully developed and can be differentiated in both the living condition and in stained preparations. Likewise, in the estivo-autumnal infections certain bodies are developed, of typical crescentic shape, the so-called "crescents," that only undergo development within the mosquito. These bodies while in the blood of man are known as *gametes* and are sexually differentiated, the male being known as the *microgametocyte* and the female as the *macrogametocyte*. When they reach the stomach of the mosquito the male, or *microgametocyte*, liberates flagella, which are known as *microgametes*, and which serve to fertilize the female *macrogametocyte*, which, after certain maturation phenomena, is now known as the *macrogamete*. This process may rarely be observed in preparations of blood removed some time from the body if a little moisture be present on the slide and the temperature be suitable. The fertilization of the *macrogamete* occurs normally in the middle intestine of the mosquito and the result of the fertilization is known as the *sporont*. After a certain period of time the *sporont* elongates and becomes motile and is then known as the *ookinete*. The *ookinete* penetrates the wall of the middle intestine of the mosquito and eventually becomes situated between the adipose tissue and the muscular wall of the intestine. Here the organism becomes circular in shape and forms a cyst known as the *oöcyst*. At this stage the cytoplasm is reticular and granular in appearance, the pigment is reduced in amount, and the entire organism is inclosed in a well-defined capsule. The *oöcyst* is formed at about the third or fourth day after infection of the mosquito. About the fifth or sixth day the *oöcyst* enlarges and within it are formed spherical refractive bodies known as *sporoblasts*. At this stage the organism is so greatly increased in size that it projects from the intestinal wall and may be seen with a low-power objective. At the end of a week the *sporoblasts* have produced a large number of delicate filaments having pointed extremities and containing a small amount of chromatin, which are called *sporozoites*. They are about 14 microns in length and are arranged in a ray-like formation about a central mass in the *sporoblast*, which may contain pigment. The *sporozoites* are finally liberated in the body-cavity of the mosquito by the rupture of the cyst and make their way to the tubules of the salivary glands. At this time, if the infected mosquito bites a man, the *sporozoites* will be inoculated, taken up by the blood stream, and penetrating the red blood corpuscles, develop into *schizonts* and thus begin the human life cycle of plasmodia. The entire life cycle in the mosquito varies from 10 to 14 days in duration.

Having thus briefly reviewed the mosquito-cycle of the malaria plasmodia, we will now consider the morphology of those forms

which occur in the blood of man and which are intended to complete their development in the mosquito.

Morphology of the sexual forms of the plasmodia occurring in the blood of man.—The forms of the malaria plasmodia concerned in *sporogony*, or the mosquito cycle of development, which can be demonstrated in the blood of the human host are of the greatest importance in the prophylaxis of these infections, and their recognition essential in any scientific campaign against malaria. The

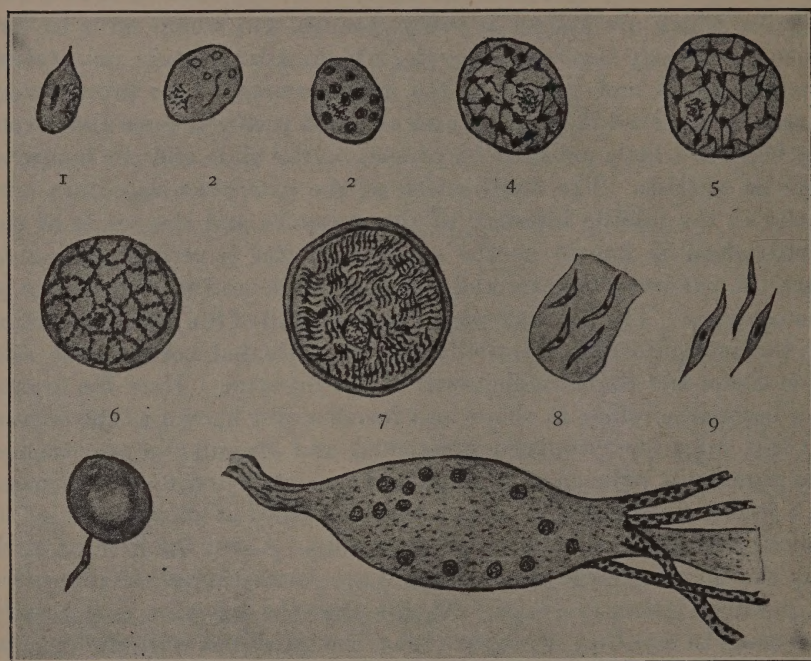


FIG. 1.—Development of *Plasmodium vivax* within the mosquito. 1, Ookinete; 2, oocyst; 3, 4, 5, oocysts showing the development of sporoblasts; 6 and 7, oocyst showing development of the sporozoites, which are fully formed in 7; 8, sporozoites within a cell of the salivary gland; 9, sporozoites; 10, entrance of sporozoite into a red blood corpuscle; 11, middle intestine (stomach) of mosquito, showing several oocysts in the wall of the organ. (Original.)

presence of these sexual forms proves beyond question that the individuals in whom they occur are infective to mosquitoes, and that they are thus true "carriers" of malarial disease. The proper treatment of these "carriers" is thus dependent upon the differentiation of the sexual forms or *gametes* and no malaria survey can be considered complete unless the percentage of individuals showing *gametes* is ascertained and proper measures be taken to render them harmless. As already stated, the *gametes* are sexually differentiated, the male being called a *microgametocyte* and the female a *macrogametocyte*,

but for convenience of description I shall refer to them as the male and female *gamete* respectively.

While no true development of the *gametes* occurs within man, if blood containing them be removed from the body, the male *gamete* frequently undergoes flagellation and liberates the *microgametes*, a process normally occurring in the middle intestine of the mosquito. In very rare instances the fertilization of the female *gamete* by one of these *microgametes* may be observed in the blood after removal from the body, but this can only be regarded as an accidental occurrence, as it normally occurs only within the infected mosquito. The differentiation of the sexual forms or *gametes* is not a very difficult matter, although the opposite opinion appears to be generally prevalent. The estivo-autumnal *gametes*, because of their *crescentic* shape when fully developed, are easily recognized, even by a novice in malarial parasitology, but the intracorpuseular stages in the development of the *gametes* of all of the species of plasmodia are more difficult of recognition, although with a clear knowledge of their morphology and a little practice all of the stages of development may be differentiated in stained preparations of blood. The fully developed *gametes* of *Plasmodium vivax* (the tertian plasmodium) and of *Plasmodium malariae* (the quartan plasmodium) are easily differentiated if one is acquainted with their morphology.

In considering the morphology of the *gametes* it will be necessary to describe that of each species, as observed in both fresh and stained specimens of blood. The description given will include only the salient diagnostic points, and will be found true of the vast majority of organisms, although frequent deviations will be observed due to artefacts produced during the staining process or to developmental anomalies brought about by adverse conditions in the human host. (The stain used was Wright's modification of the Romanowsky method, already described.)

The gametes of Plasmodium vivax (the tertian plasmodium).
Fresh preparations.—In fresh preparations it is practically impossible to distinguish the *gametes* of *Plasmodium vivax* from the forms of the human life cycle until they are fully developed. While individual *gametes* may contain a larger amount of pigment, which is coarser in structure, this distinction can not always be made. However, when the *gametes* are fully developed they may be easily distinguished, even when unstained, from the fully developed forms of the human cycle, and the male and female forms can be readily differentiated.

The male Gamete or Microgametocyte.—The living *microgametocyte* of *Plasmodium vivax*, when fully developed, measures from 8 to 10 microns in diameter, and almost fills the infected red corpuscle; it is spherical in shape and is more or less filled with dark

brown pigment collected in small clumps throughout the cytoplasm. The pigment may be very motile, especially just before exflagellation, and this serves to distinguish it from the female *gamete*, in which the pigment is motionless.

The phenomenon which distinguishes the living *microgametocyte* from the forms occurring in the human life cycle is the process known as flagellation, during which the *microgametes* are finally extruded and liberated from the parent organism. The *gametes* that are about to undergo this change are easily recognized because of the violent activity of the pigment within them, and the undulatory movements of the periphery of the parasite, due to the flagella or *microgametes* moving about within the organism. If such a parasite be watched it will be observed that eventually a number of delicate filaments suddenly make their appearance at the periphery of the organism and lash about in the blood plasma. After a variable time one, or perhaps all, of the flagella or *microgametes* succeed in freeing themselves from the parent body and disappear among the red-blood corpuscles. Sometimes these free *microgametes* are discovered apparently alone in a blood specimen by the movement which they impart to the red corpuscles in their vicinity and have been mistaken for very minute filariæ. Their presence is sufficient to stamp the individual from whom the blood was obtained as a "carrier" of malaria.

The female gamete or macrogametocyte.—The tertian *macrogametocyte* when fully developed measures from 9 to 11 microns in diameter, is circular in shape, and almost fills the infected red cell. The cytoplasm is more granular than is that of the forms of the human life cycle, and the pigment is coarser in character and instead of being distributed throughout the cytoplasm is collected in a wreathlike manner at some distance from the periphery. The pigment is not motile. The arrangement of the pigment is characteristic and is of great value in the diagnosis of this *gamete* in living specimens.

Stained preparations.—While it requires considerable practice to distinguish the *gametes* of the tertian plasmodium from the forms concerned in the human life cycle in living specimens, it is quite easy to make the distinction in stained preparations, and in such preparations even the young *gametes* may be differentiated from the "ring-forms" of the human cycle. The staining reactions of the cytoplasm and nucleus of the malaria plasmodia have already been described, and the only variation that occurs in the staining reactions of the *gametes* consists in the degree of color imparted by the stain to the various forms. The tertian *gametes* when stained with the Wright method consist of a mass of blue cytoplasm, inclosing the

red-stained chromatin; the unstained portion of the nucleus is not always distinct, and the *gamete* is considerably larger in every stage of development than the *schizont*. The pigment is more abundant, while the nuclear chromatin shows no evidence of division and distribution throughout the cytoplasm, being arranged in small irregular masses confined to one portion of the parasite, or in fine threads or grains, surrounded by an unstained area, which in turn is surrounded by the blue cytoplasm.

The following points are of service in differentiating tertian *gametes* from the *schizonts*, or forms concerned in the human life cycle: 1. The young *gamete* is never "ringlike" in shape, as the dot of nuclear chromatin is situated *within* the circular parasite instead of at some portion of the periphery. This "bull's-eye" arrangement is characteristic, and any organism presenting it may be diagnosed as a *gamete*. 2. The *gamete* is larger than the corresponding stage in the development of the *schizont*. 3. The pigment in the *gamete* is larger in amount and earlier developed. 4. The chromatin is not distributed in the cytoplasm, but occurs in a clump surrounded by the cytoplasm. 5. *Gametes* never sporulate.

The male *gamete* or *microgametocyte* varies in appearance when stained with the stage of development. The small, intracorpuseular *gametes* stain a pale blue and contain a large dot of intensely red chromatin situated centrally. The fully developed *microgametocytes* present a very poorly stained cytoplasm, the blue tinge being so faint in many instances as to be distinguished with difficulty, the cytoplasm appearing hyaline. The chromatin is large in amount, and in those organisms about to flagellate it is collected into several masses, which are arranged about the periphery of the organism. The pigment is large in amount and stains a greenish-blue color.

The chromatin in the *microgametocytes* is always larger in amount than in the female *gamete* or *macrogametocyte*, and is arranged in the form of rather thick fibrils collected in irregular masses in the cytoplasm, but all of them surrounded by a single achromatic zone, except just prior to exflagellation.

The female *gamete* or *macrogametocyte* of the tertian plasmodium stains a very intense blue, and this alone serves to distinguish it from the male *gamete* or the *schizont*, neither of which stain so intensely. The chromatin in the smallest forms appears as a single red dot situated near the center of the organism, while in later stages of development several dots or rods of this substance are present, situated near the periphery and surrounded by an achromatic zone. The chromatin is never distributed in the cytoplasm as in the *schizonts* or in the male *gamete* just prior to flagellation. The pigment is

almost black in color, occurs in the form of minute rods, and is arranged in irregular masses near the periphery or in a wreath about the center of the organism.

The tertian *microgametocyte* may be distinguished from the *macrogametocyte*, in stained specimens, by the pale blue staining of the cytoplasm as compared with the deep blue of the *macrogametocyte*, the larger amount of chromatin, collected in masses and surrounded by an achromatic zone, and the presence of several irregular masses of chromatin, situated near the periphery, when the organism is about the flagellate. In the young *gametes* it is impossible to distinguish between the male and female forms, except by the staining reaction of the cytoplasm, the male staining a very pale blue, while the female stains a deep blue.

The gametes of Plasmodium malariae (the Quartan Plasmodium).—The gametes of *Plasmodium malariae* are very similar to those of *Plasmodium vivax*, which have just been described, and for this reason require no extended description. Allowing for the difference in the size of the two species, the staining reactions, and the differentiation of the male and the female forms are practically identical, and the description of one can be used for the other if it be remembered that the quartan *gametes*, at every stage of development, are much smaller than the tertian.

The gametes of the estivo-autumnal plasmodia.—The sexual forms, or *gametes*, of *Plasmodium falciparum* and *Plasmodium falciparum quotidianum* are commonly known as “crescents,” and are easily distinguished from any other form of plasmodium. The presence of these crescents in the blood at once stamps the individual as a “carrier” of malaria, but the early stages of the estivo-autumnal *gametes* are not crescentic in shape, and at this time it is impossible to differentiate them from the quartan *gametes*, as they are similar in structure and in size. When fully developed the crescents are easily recognized, and the male and female forms can be differentiated with little trouble.

What has been said regarding the appearance of *gametes* of tertian malaria in the earlier stages of development is equally true of the *gametes* of the estivo-autumnal plasmodia, and it is only after they have assumed the crescentic shape that they can be said to differ in morphology from the tertian or quartan *gametes*, if we except their size, which is slightly smaller than the quartan and much smaller than the tertian *gamete*. It will not be necessary, then, to describe the early stages of development of these bodies, but only their morphology after the typical crescentic shape has been acquired. This occurs while the parasites are still within the infected red corpuscle, and as the organism gradually enlarges the infected cell shrinks about it, forming an envelope, which is most

noticeable between the poles of the crescent, where it forms what is known as the "bib" of the crescent.

As the *gametes* of the tertian and quotidian estivo-autumnal plasmodia are identical in morphology, except that the quotidian *gametes* are considerably smaller than the tertian, they will not be described separately, and only the points of diagnostic importance will be touched upon in the description of these stages of development.

In the *living* condition the estivo-autumnal *gametes*, after reaching the crescentic stage, are distinguished by their typically crescentic outline and by the fact that they appear to be extracellular, in the vast majority of instances. The cytoplasm appears granular in structure, the female form being more granular than the male, and the pigment is dark brown in color and either distributed throughout the cytoplasm or collected near or at the center of the crescent. The

EXPLANATION OF PLATE 7.

PLATE 7.

FIGURE 1.—Phagocytosis of sporulating *Plasmodium falciparum*. Wright's stain. $\times 1800$.

FIGURE 2.—*Plasmodium falciparum*. A *macrogametocyte*. The female crescent or gamete. Wright's stain. $\times 1200$.

FIGURE 3.—*Plasmodium falciparum*. A *macrogametocyte*. The female gamete or crescent. Wright's stain. $\times 1500$.

FIGURE 4.—*Plasmodium falciparum*. A *microgametocyte*. The male gamete or crescent. Wright's stain. $\times 1200$.

male crescent, or *gamete*, is easily distinguished from the female by its plump kidney-like shape, the female crescent being long and slender. These bodies are very easily recognized in unstained specimens of blood and in regions where estivo-autumnal malaria is endemic a considerable proportion of the inhabitants will be found to show them in their blood.

In *stained* preparations the crescents present the same staining reactions as do the *gametes* of the tertian and quartan parasites, the male crescent staining a pale blue while the female stains intensely. The following features serve to distinguish the estivo-autumnal *microgametocytes* (males) from the *macrogametocytes* (females):

1. Shape: The plump kidney shape, the *macrogametocyte* being long and slender.

2. Staining reaction: The pale blue staining of the cytoplasm, the *macrogametocyte* staining deep blue.

3. Chromatin: The arrangement of the nuclear chromatin in the form of a loose network, the chromatin of the *macrogametocyte* being situated at the center of the crescent in a dense mass.

4. Pigment: The distribution of the pigment throughout the cytoplasm, the pigment of the *macrogametocyte* being concentrated at or near the center.

5. Size: The *microgametocytes* are shorter and broader than the *macrogametocytes*.

I do not consider it necessary to describe more minutely the morphology of the estivo-autumnal *gametes*, as their crescentic shape is sufficient to distinguish them in either fresh or stained preparations of blood, and as their recognition is only of importance in malaria prophylaxis, this end will be attained by attention to the diagnostic points already mentioned. For a more detailed description the reader is referred to any of the modern works dealing with the malarial fevers.

It should be remembered that after the crescents reach the middle intestine of the mosquito the crescentic shape is lost; they become circular in outline, the male crescent flagellates, and the flagella, or *microgametes*, are liberated and fertilize the female, as in the tertian and quartan parasites, after which a similar cycle of development occurs in the mosquito. The process of flagellation is often observed in blood removed some time from the body, especially if a little moisture is present upon the microscopic slide.

Important differential points in the diagnosis of the species of malaria plasmodia.—The various species of malaria plasmodia may be easily differentiated in both fresh blood and in stained preparations if certain morphological peculiarities of the plasmodia and the cells infected by them be remembered. The following points I have found most useful in differentiating the various species:

A. *Plasmodium vivax* (tertian plasmodium).

Unstained preparations.—1. Comparatively large size of parasite after development of pigment. 2. Enlargement of the infected red blood corpuscle. 3. Active ameboid motility. 4. Fine, light-brown or reddish-brown pigment. 5. Number of spores or *merozoites*, 12 to 24. 6. All stages of development present in the peripheral blood.

Stained preparations.—1. Large size, after development of pigment. 2. Increased size of the infected erythrocyte. 3. Presence of Schuffner's dots (eosinophilic granules) in cytoplasm of infected erythrocyte. 4. Number of *merozoites*, 12 to 24. 5. Presence of all stages in the peripheral blood.

B. *Plasmodium malariae* (quartan plasmodium).

Unstained preparations.—1. Medium size, after development of pigment. 2. Pigment in coarse, dark-brown granules, arranged about periphery of organism. 3. Infected erythrocyte not enlarged at any stage of development. 4. Presence of all stages in peripheral blood. 5. Number of *merozoites*, 6 to 12.

Stained preparations.—1. Medium size of pigmented organisms. 2. No increase in the size of the infected red blood corpuscle. 3. Absence of Schuffner's dots (eosinophilic granules) in cytoplasm of infected cell. 4. Number of spores (*merozoites*), 6 to 12. 5. Presence of all stages in the peripheral blood. 6. Presence of the "band forms."

C. *Plasmodium falciparum* (tertian estivo-autumnal plasmodium).

Unstained preparations.—1. Small size, even when fully developed. 2. No enlargement of infected corpuscle, which is smaller than normal. 3. Small amount of fine brownish pigment. 4. Number of *merozoites*, 10 to 16. 5. Only ring forms and young pigmented forms present in the peripheral blood, and crescents. 6. Crescentic shape of the *gametes*.

Stained preparations.—1. Small size, even the sporulating forms only partly filling the infected cell. 2. No enlargement of the infected corpuscle. 3. Presence of Maurer's dots or basophilic granules in the cytoplasm of the infected cell. 4. Number of *merozoites*, 10 to 16, as a rule, very small and arranged irregularly. 5. Only "ring-forms" and young pigmented forms occur in the peripheral blood, as a rule, together with crescents after the infection has persisted for several days. 6. Crescentic shape of the *gametes*.

D. *Plasmodium falciparum quotidianum* (quotidian estivo-autumnal plasmodium).—The quotidian estivo-autumnal plasmodium differs from the tertian form, in both fresh and stained preparations, by its smaller size at every stage of development, while the *merozoites* number from 6 to 18, the average being from 12 to 14. The organism contains less pigment, which is collected into one or two almost black masses, and the sporulating forms seldom fill more than one-third of the infected red blood corpuscles. The hyaline "ring-forms" are very minute, resembling in this respect the piroplasma.

Objects which may be mistaken for plasmodia in stained and unstained specimens of blood.—There is no one thing more essential to success in the recognition of the malaria plasmodia than a practical knowledge of the microscopical appearance of both fresh and stained specimens of normal and pathological blood. It is surprising how often some artefact or other object is mistaken for a malaria plasmodium by medical men from whom such a mistake would not be expected, and it is always due to ignorance of the morphology of the blood, an ignorance that can not be excused, for the blood "we have always with us" and anyone may become perfectly familiar with its morphology who cares to take the time to make a few preparations and to study them. Mistakes are more apt to occur in examining fresh blood but a little care and practice will enable one to avoid them and with the excellent Wright stain it is inexcusable to mistake any object for a plasmodium in stained preparations.

In *fresh* specimens of blood the following may be mistaken for malaria plasmodia:

Vacuoles.—There is no object more deceiving in fresh blood preparations than a small vacuole within the red corpuscle, especially if the border of the vacuole alter in shape, when the resemblance to the hyalin disk forms of the various plasmodia is really very striking. The greater refraction of the vacuole and the fact that it grows larger and smaller in focussing up and down upon it should distinguish it from any stage in the growth of the malaria plasmodia.

Retraction of the hemoglobin from the edge of the red corpuscle may sometimes cause the area devoid of hemoglobin to resemble a hyalin plasmodium, but it may be distinguished by the lack of ameboid motion and the sharp edge of the hemoglobin layer.

"Eye-spots," or areas devoid of hemoglobin, occurring within the red corpuscles are sometimes observed in normal blood, and frequently in typhoid fever, tuberculosis, pneumonia, and other acute infectious diseases. They are apparently due to retraction of the hemoglobin layer of the corpuscle, and may be round, oval, spindle-shaped, or ring-shaped, and situated at any portion of the corpuscle. Many of them are oval and spindle-shaped and contain a dark area at the center resembling pigment. These "eye-spots" have been very often mistaken for malaria plasmodia and have been described by several observers as new blood parasites of man. The absence of distinct ameboid motion and the greater degree of refraction should serve to distinguish them from malaria plasmodia.

Blood platelets.—A blood platelet, if lying upon a red corpuscle, resembles slightly the hyalin disk form of malaria plasmodia, but upon focussing there will be observed a difference in the level of the two bodies, while the platelet has no ameboid motility and is less opaque than the plasmodia.

Granules derived from broken down leucocytes, which possess active Brownian movements, may be confused with the smallest forms of plasmodia if they become attached to, or lie over, the red blood corpuscles. Their smaller size and different structure should render such a mistake impossible.

The normal *lighter colored center* of the red corpuscle, which is often hyalin in appearance in diseases accompanied by anemia, has often been confused with the hyalin malaria plasmodia, and I have several times been shown cells of this character and told that they contained plasmodia. Such a mistake is due to inexcusable ignorance of the morphology of the red blood corpuscle.

Crenations of the red corpuscle may cause some trouble to the beginner if they be looked down upon directly, as they then appear as round, hyalin bodies lying upon the red cell, and are sometimes slightly motile. However, there is no ringlike outline, and when

focussed upon they appear as alternately light and dark areas upon the surface of the red corpuscle.

Among other bodies that have been mistaken, in my experience, for malaria plasmodia, and shown to me, may be mentioned yeast cells, bacteria of various kinds, extraneous matter, and degenerating red blood corpuscles possessing slender cytoplasmic processes. The latter have been mistaken for flagellating plasmodia, but the greenish color and lack of pigment should prevent such a mistake.

In specimens of blood *stained* by Wright's method the following have been mistaken for malarial plasmodia:

Blood plates.—There is no object so frequently mistaken for malaria plasmodia in stained preparations of blood as is the blood platelet, especially when it lies upon a corpuscle, but such an error is impossible if one is familiar with the morphology of the platelet. The malaria plasmodia of a similar size may always be differentiated from the platelets by their blue-stained cytoplasm and the solid, compact mass or masses of chromatin which stain a ruby red color. The chromatin of the blood platelet stains a darker color and is distributed throughout the platelet in the form of fine granules or irregular granular masses. If one remembers that any malaria plasmodium the size of a blood platelet consists only of a mass of blue-stained cytoplasm containing a solid spherical dot, or at most two dots, of chromatin, it will be impossible to confuse it with a blood platelet, which has a practically unstained cytoplasm and in which the chromatin is granular and collected in a mass or masses in the cytoplasm. Not frequently blood platelets may be collected in crescentic shaped clumps and these have been mistaken for crescents, but no one in the least familiar with the plasmodia could make such a mistake.

Flaws in the microscopic slide, in which some of the stain has settled, have caused confusion, especially if overlaid by a red-blood corpuscle, but focussing will show that they are on a different level from the corpuscle, and a study of the staining reaction will immediately demonstrate their nature.

Vacuoles in the red cell, which sometimes retain a little of the stain, may slightly resemble some of the stages of development of the plasmodia, but may be easily distinguished from the plasmodia by the absence of the blue-stained cytoplasm and red chromatin always present at every stage in the development of the malaria parasites.

Among other objects which have been confused with the plasmodia in stained smears may be mentioned degenerating erythrocytes and leucocytes, yeast cells, and other microorganisms, nucleated erythrocytes, basophilia of the erythrocyte, and extraneous bodies of various nature. It is perfectly obvious that a knowledge of the appearance

of normal and pathological blood in stained preparations will make such mistakes impossible, and this is absolutely essential to one who expects to make microscopic examinations of the blood for the diagnosis, and as an aid in the prophylaxis, of malarial infections. In the military service our entire system of prophylaxis and treatment of malaria should be based upon the recognition of the malaria plasmodia in man and the mosquito, for in this service the material is under absolute control and such a basis for prophylaxis and treatment is possible.

CHAPTER II.

THE MALARIA MOSQUITOES.

The Culicidæ, or mosquitoes, belong to the true flies, or Diptera, and the only insects of this family of interest to us in the prophylaxis of malaria are some of the species belonging to the subfamily *Anophelinae*, for it is only species belonging to this subfamily that transmit malaria. In this section only those points of interest or value to the student of malaria prophylaxis will be touched upon, no attempt being made to give a complete account of either the anatomy or life history of the mosquitoes, as a whole, or to give descriptions of the species concerned in the transmission of the malarial infections. For a complete discussion of the subject the reader is referred to works of Theobald⁵, Howard, Dyar, and Knabe⁶, and Ludlow⁷.

Geographical distribution of the Anophelinae.—Mosquitoes belonging to the *Anophelinae* occur wherever malaria does, but it does not follow that malaria is always present in localities where the *Anophelinae* are found. In order that malarial infections be present in any locality, the right species of *Anophelinae* must be present, together with infected individuals, and unless both factors be present the propagation of these fevers is impossible. It may be stated that the geographical distribution of the malaria mosquitoes coincides with the distribution of malaria, and the amount of malaria in any given locality is an index of the number of the *Anophelinae* present.

This fact is of great importance in the prophylaxis of the disease, as the determination of the presence of anophelines and of the exact species present enables one to avoid localities inhabited by the malaria mosquitoes or to take the proper precautions against infection. Such knowledge is of special value in the military service, as a preliminary "mosquito survey" in the selection of localities for camp sites or for the sites of military posts will often prove of inestimable value, both as regards the health of the troops which occupy them and the cost of maintenance. Several instances have occurred in which this measure was neglected, with the result that the camps or posts so located became hotbeds of malaria and a source of continued and excessive expense, owing to the necessity of maintaining costly prophylactic operations. Where there is no military reason for occupying a malarial locality as a camp site, or as a permanent post, it is the part of wisdom to avoid doing so, and the determination of the

species of mosquitoes which may be present and the feasibility of getting rid of them should be as carefully investigated as the character of the water supply, the terrain, or any other feature of the locality.

The *classification* of mosquitoes, as regards genera and species, is still an unsettled question among entomologists, but in the following pages I shall follow that of Theobald, who divides the *Anophelinæ* into several genera, each containing several species. Of the genera given by Theobald, the following contain species that have been proven to transmit malaria: *Anopheles*, *Myzomyia*, *Stethomyia*, *Pyretophorus*, *Arribalzagia*, *Myzorhynchus*, *Nyssorhynchus*, and *Cellia*.

For convenience of reference I have prepared the following table, giving the geographical distribution of the species of mosquitoes which have been proven to transmit the malarial fevers. The table can not be considered as complete, as observations are being continually published demonstrating the transmission of these infections by hitherto unsuspected species of the *Anophelinæ*, but it will serve as a guide to the commonly observed species which transmit malaria in the regions mentioned.

Locality.	Species of malaria mosquitoes.
United States.....	<i>Cellia albimanus</i> , <i>Cellia argyritarsis</i> , <i>Anopheles maculipennis</i> , <i>Anopheles quadrimaculatus</i> , <i>Anopheles crucians</i> , <i>Anopheles intermedium</i> , <i>Anopheles pseudomaculipes</i> , <i>Anopheles pseudopunctipennis</i> , <i>Anopheles tarsimaculata</i> .
West Indies.....	<i>Cellia albimanus</i> , <i>Cellia argyritarsis</i> .
Canal Zone, Panama.....	<i>Cellia argyritarsis</i> , <i>Cellia albimanus</i> , <i>Anopheles pseudopunctipennis</i> , <i>Anopheles tarsimaculata</i> .
Philippine Islands.....	<i>Myzomyia funesta</i> , <i>Myzorhynchus sinensis</i> , <i>Myzorhynchus barbirostris</i> , <i>Myzorhynchus fuliginosus</i> , <i>Myzomyia ludlowii</i> ?
Central and South America....	<i>Anopheles albipes</i> , <i>Pyretophorus lutzii</i> , <i>Cellia argyritarsis</i> , <i>Cellia albimanus</i> , <i>Anopheles pseudomaculipes</i> , <i>Anopheles intermedium</i> , <i>Anopheles cruzii</i> .
Europe.....	<i>Anopheles maculipennis</i> , <i>Anopheles bifurcatus</i> , <i>Anopheles superpictus</i> , <i>Myzomyia hispaniola</i> , <i>Myzorhynchus pseudopictus</i> .
Asia.....	<i>Myzomyia culicifacies</i> , <i>Myzomyia listoni</i> , <i>Myzomyia turkhudi</i> , <i>Myzorhynchus barbirostris</i> , <i>Myzorhynchus sinensis</i> , <i>Nyssorhynchus theobaldi</i> , <i>Nyssorhynchus stephensi</i> , <i>Nyssorhynchus fuliginosus</i> , <i>Nyssorhynchus maculipalpis</i> , <i>Pyretophorus jeyporensis</i> .
Africa.....	<i>Myzomyia funesta</i> , <i>Myzomyia nili</i> , <i>Myzorhynchus barbirostris</i> , <i>Myzorhynchus paludis</i> , <i>Cellia pharoensis</i> , <i>Pyretophorus costalis</i> , <i>Pyretophorus chaudoyci</i> .

If one desires to follow the classification of Howard, Dyar, and Knab, the generic names in the above table should all be replaced by the generic name, *Anopheles*.

The number of infected mosquitoes in malarial regions.—It is obvious that the number of mosquitoes infected in different localities will vary with the number of infected individuals present, the season of the year, certain atmospheric conditions, and the care observed by the inhabitants in protecting themselves from the bites of mosquitoes. At the present time we have no evidence that the infection in the mosquito is hereditary, so that it follows that every infected mosquito must have become infected from a human being, and one infected individual may infect scores of mosquitoes within a few

hours. In practice it has been found that even in the worst malarial regions a surprisingly small percentage of mosquitoes examined are found infected, if one considers the actual number of infected individuals present and the apparent chance of infection of the mosquito.

In Italy Celli found only 2.5 per cent of the *Anopheles* examined by himself infected; A. Plehn, in Africa, examined 860 *Anopheles* and found 2.2 per cent infected; La Monaco found about 5 per cent infected; and the Sergeants, in Algiers, found 1.6 per cent infected. At Camp Stotsenburg, in the Philippine Islands, the percentage of naturally infected mosquitoes varied greatly, being as high as 35 per cent during the height of the malarial season and as low as 1 per cent at other times.

The species of mosquito in relation to the transmission of malaria.—It is now a well-established fact that certain of the *Anophelinae* do not transmit malaria and can not be infected experimentally. The observations of Stephens and Christophers in India showed that while 12 of 259 *Myzomyia culicifacies* dissected by them shown infection with malarial *sporozoites*, not a single one of 496 *Myzomyia rossii* showed infection, while Hirshberg found that out of 58 *Anopheles punctipennis* experimentally none developed an infection, while of 48 *Anopheles maculipennis* 8 became infected. In the Canal Zone Darling⁸ found that, in his experiments, 70.2 per cent of *Cellia* (*Anopheles*) *albimanus* became infected; 60 per cent of *Anopheles tarsimaculata*, and 12.9 per cent of *Anopheles pseudopunctipennis*. On the other hand, he found it impossible to infect *Anopheles malefactor*, which, as he says, despite its name, is not a factor in the transmission of malaria in the Canal Zone.

From these observations it is evident that even though anophelines may be present in a locality, it does not follow that malaria is present or that the disease could spread if it were introduced. In other words, the right species of *Anopheles* must be present in order to transmit the infection, and hence it follows that the determination of the species present in any locality becomes an important prophylactic measure.

Not only are some of the *Anophelinae* unable to act as hosts of the malaria plasmodia but some species can only act as host for a certain species of plasmodium, so that the prevalence of the different types of malaria in any locality depends upon the species of mosquito present. Beyer, Pothier, Couret, and Leman⁹ have shown that while *Anopheles quadrimaculatus* became infected experimentally with the tertian and quartan plasmodia it was impossible to infect it with the aestivo-autumnal plasmodium, while, on the other hand, *Anopheles crucians* was shown to be a host to the aestivo-autumnal plasmodium but could not be infected with the tertian or quartan plasmodia. Kinoshita¹⁰ found that *Anopheles*

sinensis could be infected with the plasmodia of tertian and quartan malaria but not with those of aestivo-autumnal malaria, and that the quartan plasmodium only developed in this species of mosquito at low temperatures. He also has shown that *Anopheles listoni* is the great transmitter of the aestivo-autumnal plasmodia in Formosa. Many observers have proven that the common *Anopheles maculipennis* is an efficient host for all of the species of malaria plasmodia.

The observations noted explain clearly why certain localities suffer more severely than others from certain types of malarial infection. Given a locality in which only *Anopheles crucians* occurred and we would have nothing but aestivo-autumnal infections, as this mosquito is unable to transmit the tertian or quartan plasmodia, but if *Anopheles quadrimaculatus* were the only anopheles present, we might have either tertian or quartan infections but no aestivo-autumnal malaria. It is, therefore, evident that a mosquito survey is not only of value in determining whether dangerous species of *Anopheles* are present or not, but that it will also aid us in estimating the probability of the spread of the various types of malarial infection.

General description of the Anophelinae.—The following brief description of the anatomy of the *Anophelinae* only includes the points of value in the differentiation of these mosquitoes or that are essential to a clear understanding of the relation that these insects bear to the transmission of malaria.

External anatomy.—All mosquitoes are divided into three well marked areas for purposes of description: the head, the thorax, and the abdomen.

The *head* presents upon each side a prominent compound eye, the space separating the eyes above being known as the *occiput*, and that separating the eyes in front as the *vertex*; the back of the head is called the *nape* or neck. The structures of greatest importance from a diagnostic standpoint attached to the head are the *antennae* and the *palpi*, for attention to these structures alone is generally sufficient to enable one to distinguish between the *Anophelinae* and other mosquitoes. The *antennae* are two jointed structures arising from the head and vary in length and structure. In the male they are plumose but in the female they are surrounded by hairs, in most species, so that the sex of a mosquito is easily determined by noting whether the *antennae* terminate in minute plumes or whether they are surrounded by hairs that become shorter as the end of the antenna is reached. As only the females suck blood the determination of the sex is of importance in any study of the prevalence of malaria.

The *palpi*, two in number, arise from the head between the *antennae* and the *proboscis*, and are of value in the differentiation of

the *Anophelinae*, as in this group the *palpi* are of practically the same length as the *proboscis* and longer than the *antennae*, while in *Culex* and *Stegomyia* the *palpi* in the female are short, club-like structures, very much shorter than the *proboscis* or *antennae*. As a practical working rule it may be stated that any female mosquito having *palpi* approximating the length of the *proboscis* belongs to the *Anophelinae* and, therefore, may be concerned in the transmission of malaria.

The *proboscis*, or sucking and piercing organ, is a complex structure arising from the head between the *palpi*. It consists of seven parts, the labrum, the epipharynx, the hypopharynx, two mandibles or lancets, and two maxillae, which, when the insect is not biting, are all inclosed in the labium. All of the mouth parts originate beneath the *clypeus*, a chitinous prolongation of the anterior portion of the head. At the base of the *hypopharynx* is situated the so-called *salivary pump* in which the salivary duct empties and is continued beyond it into the *hypopharynx*. The action of this pump is to draw the saliva from the salivary glands into the pumping organ after which it is forced into the channel along the hypopharynx. The saliva is injected into the wound when the mosquito bites and with it the malarial *sporozoites* if the insect is infected.

The thorax.—The thorax is situated between the head and abdomen and is composed of the *mesothorax*, the *scutellum*, the *prothoracic* lobes, and the posterior portion, or *metathorax*. It contains the salivary glands and is of importance to entomologists in the classification of mosquitoes, because of the character of the scales which cover it. The *legs* arise from the thorax, and are six in number, and the two *wings* arise from the upper portion of the thorax and their venation is of great importance in classification.

The abdomen.—The abdomen consists of 10 segments, the posterior two being much smaller than the others and containing the sexual organs. The scales that cover the abdomen in most species are of considerable value in classification to the systematic entomologist, as they vary greatly in shape and arrangement.

The internal anatomy of the Anophelinae.—The alimentary canal of mosquitoes is divided into a *fore-gut*, a *mid-gut*, and a *hind-gut*. The *fore-gut* lies in the head and thorax and consists of the mouth, the pharynx, and pumping organ, the esophagus and the esophageal diverticula. The *mid-gut* begins in the thorax and extends from the esophageal diverticula to the hind-gut, filling most of the abdominal cavity. It consists of the so-called stomach and the pylorus. The portion of the mid-gut known as the "stomach" is of special interest to the student of malaria prophylaxis, for it is in this portion that the mosquito cycle of the malaria plasmodia begins, the *ookinete* produced by the fertilization of the female plasmodium

by the male penetrating the wall of this portion of the intestinal canal and forming the cyst in which are produced the *sporozoites*. This portion of the mid-gut lies at about the level of the sixth abdominal segment.

The *hind-gut* begins at the pyloric dilatation into which open the five Malpighian tubes and ends at the anus. It consists of the pyloric dilatation, the ileum, the colon, and the rectum.

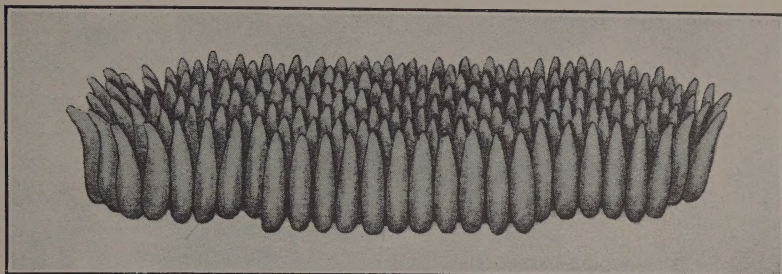
The *genital organs*, while of great importance to the entomologist in the classification of species, will not be described, as they can not be used for this purpose by the ordinary observer.

The salivary glands.—The salivary glands are situated in the anterior portion of the thorax and are of interest because it is through them that the malaria *sporozoites* reach the mosquito's proboscis and eventually the blood of man, being injected into the wound when the insect bites. The glands are two in number, each consisting of acini lined with large granular cells and possessing a central duct which unite, after leaving the acini, to form a common salivary duct which enters the alimentary canal close to the base of the proboscis. It is in the cells lining the tubular salivary glands that the malaria *sporozoites* are found at the termination of the cycle of development of these parasites in the mosquito.

The life-cycle of the Anophelineæ.—The *Anophelineæ*, in common with all mosquitoes, passes through larval and pupal stages before development into the perfect insect and these stages are aquatic so far as we know.

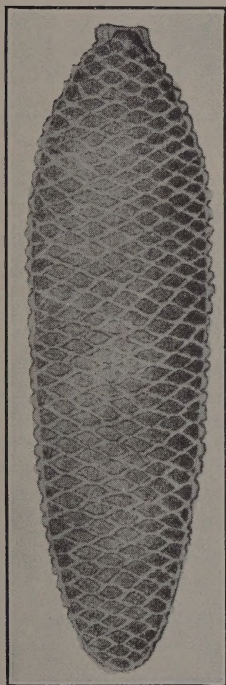
The ova.—The ova of *Anopheles* are generally laid upon the surface of water but a few species will lay them in wet ground, as I have observed in the Philippines. The eggs are not laid in boat-shaped masses, as in the case of the common *Culex* mosquitoes, but singly, and by adhering end to end geometrical patterns are sometimes produced that are quite characteristic. The distinguishing mark of the eggs of the *Anophelineæ* is the presence of the so-called "float," a hydrostatic organ composed of a partial envelope expanded along the middle of the egg, giving it a very characteristic appearance. These lateral floats are variously marked in the eggs of different species and vary in size but are always easily distinguished and of the greatest service in differentiating the anopheline egg from that of other mosquitoes. The eggs are oval in shape and eggs hatch, in most species, in from one to two days, but this varies so much with external conditions and with the different species that a general statement is hardly justified. From thirty to over a hundred eggs may be deposited by a single female.

The eggs will remain alive in moist mud for considerable periods of time, even as long as 6 days, but can not resist complete drying for over 12 hours, so far as is known. This fact is important, as it indi-



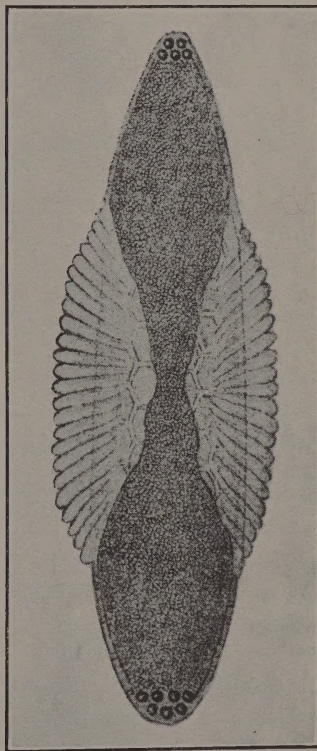
Egg raft. *Culex territans* Walk.

After Howard



After Howard.

Egg. *Stegomyia fasciata*.



After Howard.

Egg. *Anopheles crucians*.

cates that drainage of pools or other small collections of water will not be efficient if the mud remains moist until water again gains access to the pool. The observations of Nuttall and Shipley¹¹ and of Christophers and Stephens,¹² as well as others, prove conclusively that the eggs of the *Anophelinae* will remain alive in moist mud for days but that in dried mud they quickly perish.

The larvæ.—The larvæ of the *Anophelinae* are distinguished from the larvæ of other mosquitoes by the elongated, comparatively narrow

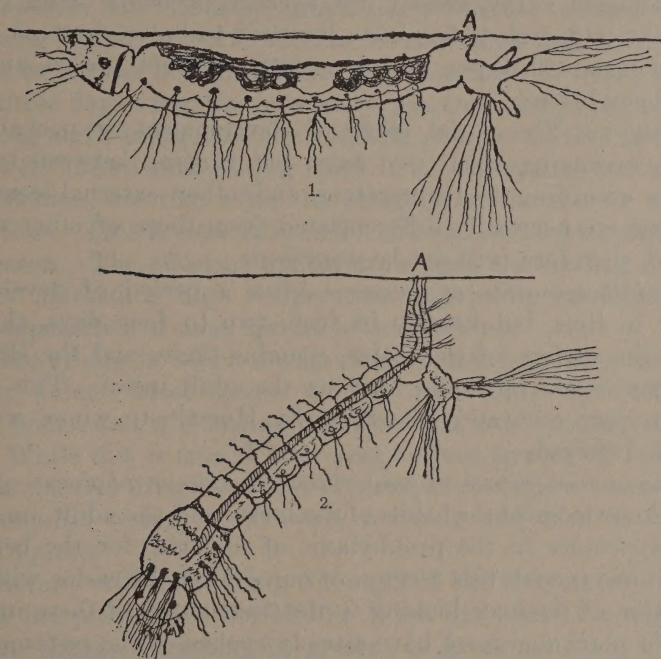


Fig. 2.—Larva of *Anopheles* and *Culex*. 1, Larva of anophelinae. Note horizontal position in relation to surface of the water, and the absence of syphon (A). 2, Larva of *Culex*. Note angular position in relation to surface of the water, and presence of the syphon, at A. (Fig. 1, after Howard; 2, after Theobald.)

head and the absence of the respiratory tube or siphon. Like the adult insect the body is divided into three portions—the head, the thorax, and the abdomen. While in the ordinary *Culex* mosquitoes the head is comparatively broad, in the anopheline larvæ it is narrow and elongated and easily distinguished. The important differential point, however, is the great difference in the breathing apparatus, the long respiratory tube of *Culex* and *Stegomyia* being replaced by openings in the eighth abdominal segment, which, while slightly raised, force the larvæ to lie parallel with the surface of the water. The long respiratory tubes of all other mosquitoes allow them, when breathing, to suspend themselves at an angle with the surface of the

water, but owing to the absence of the respiratory tube in the *Anopheles* the larvæ always lie horizontal to the surface. This fact makes the differentiation of these larvæ very easy, but, as Ludlow¹³ has pointed out, other aquatic larvæ have this peculiarity, so that one must be familiar with mosquito larvæ before concluding that anophelines are present.

The larvæ vary in color, but are usually gray or brownish. They molt four times before becoming pupæ, and the length of this stage of development varies greatly, the average probably being from 10 to 14 days, although it has been observed to be as short as a week and as long as 30 days. It varies with different species and with external conditions.

The pupæ.—The pupal stage of development is probably the shortest, averaging about two days, but varying between two and six days according to temperature and other external conditions. The pupæ are not easily differentiated from those of other mosquitoes, and, therefore, will not be described.

The adult mosquito or imago.—After a period of development varying in time, but usually in from two to four days, the pupa rises to the surface of the water, remains there, and the skin over the thorax breaks open and liberates the adult insect. This process has been very accurately described by Hurst¹⁴, to whose work the reader is referred.

Habits of mosquitoes of importance in the prophylaxis of malaria.—A knowledge of the habits of the larvæ and the adult anopheline is of importance in the prophylaxis of malaria, for the better acquainted one is with this portion of our subject the easier will be the application of methods looking to the destruction of these insects.

Habits of the larvæ.—I have already spoken of the position of the larvæ of anophelines being parallel with the surface of the water, and this position is maintained unless the larva is frightened, when it will sink below the surface. It feeds upon floating objects, which are attracted to the mouth by the fringes of the mouth parts producing a current toward the mouth. The larvæ eat both vegetable and animal matter and devour one another.

The larvæ of some species of *Anopheles* are able to *hibernate*, as shown by the observations of Galli-Valerio and de Jongh. They found that the larvæ of *Anopheles bifurcatus* hibernate beneath the leaves of aquatic plants, and that they survived even when in water between two sheets of ice.

Of the *natural enemies* of mosquito larvæ may be mentioned the fresh-water hydras, many aquatic insects, salamanders, and certain species of fish, to be hereafter considered.

Habits of the adult anopheles—Feeding.—The feeding habits of the species of anophelines that frequent the habitations of man are

of importance in prophylaxis. It may be stated that while many species of anophelines are of sylvan character, all of the species that have come in contact with man and have continued in association with him are bloodsuckers, and that, even in the sylvan species, blood is a normal food, being obtained from other animals than man. It is not true that a meal of blood is necessary to the female before depositing her eggs or, as has been stated, that the fertilization of the female is impossible unless she has sucked blood. Grassi found that *Anopheles* will bite large animals in preference to small ones—a horse being bitten in preference to a man—and that they bite in the open as well as in habitations. The first meal of blood is taken two or three days after the insect emerges from the pupa, as shown by Darling and Grassi, and they will bite at intervals of a few hours.

It is well known that during rains it is seldom that one is bitten by anophelines, but that after the cessation of the shower they are very vicious. This is especially noticeable in the Tropics during the rainy season. The effect of temperature upon the feeding habits is also very marked, a high temperature apparently stimulating the biting propensities of most species of anophelines.

The *time of day* in which the anophelines bite is an important prophylactic point. Most species of the *Anophelinae* fly during the twilight hours, and it is during this time that one is most frequently bitten. While this is true, I have seen various species of *Anopheles* biting in the late afternoon, and *Myzomyia funesta*, a common malaria mosquito, biting in the early morning and even at noon in the Philippines.

Anophelines will bite during the night if the temperature is high, as in the hot portions of the Middle West, but where the nights are cool, even though they may be present in great numbers, they bite only during the twilight hours and in the early morning. A beautiful illustration of the twilight habits of the anophelines is noted in the observations upon the Canal Zone regarding the flights of *Anopheles*, in which it was found that the flight of these insects from the propagation area to the town began about 6.30 in the evening and that it ceased before 9 in the evening, while the return flight began at 6 o'clock in the morning and lasted about half an hour.

After biting, the anophelines usually hide out of doors, within a short distance of habitations, beneath tall grass, or within the foliage of bushes and trees. However, they often remain within the habitation, and I have observed hundreds of anophelines in the daytime around the baseboards of toilet sinks and upon clothing in dark closets in the Philippines.

While, as has been said, most anophelines prefer the twilight hours for biting, they will bite at any hour of the day. Chagas and Neiva¹⁵, Smith¹⁶, and Howard¹⁷ all have recorded distances in which

biting occurred during the middle of the day, in the sunlight, but prophylactic measures based upon the twilight habit would prevent the vast majority of bites.

Flying distance.—In most of the older publications dealing with mosquitoes it is stated that they do not cover great distances in flight. This statement, in the light of our modern researches, can no longer be considered correct, even in regard to the *Anopheles*. In 1909¹⁸, I published a statement that from personal observations I believed that certain species of *Anopheles* often flew from 2 to 2½ miles in order to reach the post where the observations were made, and this statement, being so much at variance with generally received opinions regarding the flight of anophelines, was subjected to much criticism by entomologists. Since then my observations have been confirmed by workers upon the Canal Zone and by James and Christophers in India¹⁹, as well as others, and it is now well recognized that while anophelines prefer to breed near their prey, they will fly long distances in order to lay their eggs or to feed, provided there are no breeding places in the near vicinity.

The experiments of the department of sanitation in the Canal Zone in regard to the flight of anophelines are of special interest and value. In their report they say²⁰:

Adult anopheles were stained with dye and liberated at the swamp. Subsequently some of them were collected on the opposite side of the river, at the locks, and in houses 4,700 feet from the liberating station. It should be stated that the anopheles' flight was decidedly marked and was easily noted by half a dozen witnesses, when their attention had been drawn to it. Even so, not one person in areas thickly infested did note the flight until shown the way to observe it.

Regarding the flight of the anopheles the report states:

The flight was from west to east and quite marked. As it became darker the quantity of flying anopheles increased, and by looking at a dark object against a clear sky hundred of anopheles could be seen passing by.

The mosquitoes flew from the propagation area in a swamp to Gatun, and some of the breeding places were more than 2 miles from Gatun.

At Mian Mir, James found adult mosquitoes present when no larvæ could be found within 2 and 2¼ miles, and Le Prince²¹ determined that *Celia* (*Anopheles*) *albimanus*, the principal transmitter of malaria in the Canal Zone, will travel against a 4-mile breeze for distances of a mile, and possibly farther.

The confusion regarding the distance of flight of anophelines is undoubtedly partly due to observers studying different species, but while it is probably true that the majority prefer to fly short distances in order to reach their food, it must be recognized that these mosquitoes are capable of flying for two or more miles, and this fact

is of great importance in the prophylaxis of malaria. It is more than probable that much longer distances can be covered by interrupted flight, and that the anophelines are not an exception to the rule that mosquitoes will fly for great distances in order to reach their food or their breeding places.

Breeding places.—Contrary to our older notions regarding the breeding places of anophelines we now know that these mosquitoes may breed in practically any collection of water, even though there be quite a current present. Watson²², in the Federated Malay States, found that *Nyssorhynchus willmori* will breed in mountain streams where the current is swift, and that the larvæ of this species is capable of swimming against the current of these streams. As a rule the anophelines prefer to breed in comparatively clean water, as that found in slowly running streams or in pools of rain water, but they will breed in foul water and have been found in sewers. Paddy fields, storage tanks, wells, ditches, ponds, gutters, drains, and springs are all favorite breeding places of anophelines, some species preferring one place and some another. As Theobald states:

Practically all kinds of collections of water are acceptable to the larvæ; some prefer rain-water barrels, cisterns, and the water in tins, calabashes, and jam pots; others ponds, slow running streams, and along the banks of large rivers; others live in the water collected in bromelias, and in the water that collects in hollow bamboos, gaining their entrance through exit holes left by boring insects. The domestic species which are best known usually choose barrels and cisterns.

In the Philippines a favorite breeding place of anophelines was the cavity left after cutting off bamboo poles near a joint. In the latter part of the rainy season I have found larvæ in nearly every bamboo pole examined, and at Camp Stotsenburg, where temporary quarters for a large number of troops were built of this material, anophelines became a veritable scourge and malaria increased to such an extent that it was found necessary to remove the larger part of a brigade which had been stationed there.

Among the breeding places of anophelines which are most likely to be overlooked may be mentioned the small depressions in the ground produced by the hoofs of animals or the inequalities left in plowed land. In the tropics, during the rainy season, such breeding places are sometimes very numerous and furnish a considerable proportion of the mosquito population. Another source of mosquitoes generally overlooked is the water that collects at the bases of the leaves of certain plants, especially the *Bromeliaceæ*. Lutz²³ found *Anopheles cruzii* breeding in the water collected by the leaves of these plants and other observers have confirmed his results.

It has also been determined that some species of *Anopheles* will breed in brackish or salt water. Among these species may be men-

tioned *Anopheles crucians*, *Myzomyia ludlowii*, *Anopheles quadrimacutatus*, *Cellia* (*Anopheles*) *albimanus*, *Cellia* (*Anopheles*) *argyritarsis*, and *Anopheles tarsimaculata*.

From this very brief discussion of the breeding places of the *Anophelinae* it is evident that in malaria prophylaxis the practical point to remember is that no collection of water, however minute, should be considered as of no importance as a breeding place for these insects. As Smith has well said, the *Anopheles* breed "everywhere," and therefore every collection of water should be carefully examined before it is considered free from the larvæ of these insects.

Certain colors in relation to the Anophelinae.—It is a well known fact to anyone having experience with mosquitoes that they prefer dark to light colors and that a person dressed in black is much more apt to be bitten repeatedly than a person dressed in white. The researches of Nuttall and Shipley²⁴ have shown that *A. maculipennis* is very partial to certain colors, and this is probably true of other species of anophelines. They found that dark blue was the most attractive color to them and that pale green, light blue, orange, and yellow actually appeared to repel them. They call attention to the khaki uniform as especially suitable because of its lack of attraction for the insects.

Longevity and hibernation.—Our knowledge regarding the longevity of the *Anophelinae* is still incomplete, but we know that the life of the male is only a few days and that the females of different species vary greatly in length of life, some living for weeks only, while those that hibernate may live for many months.

In temperate climates the winter is passed by most anophelines in the adult stage of development, the females alone hibernating. Although this is so, the anophelines may pass through the winter in the ova or as larvæ. The females that hibernate are fertilized, and only lay their eggs in the spring, although if a warm spell occurs during the winter some of them will emerge from their hiding places and bite, and may thus cause outbreaks of malaria even in the middle of winter.

In the Tropics the adult anophelines hibernate during the hot dry season and lay their eggs at the beginning of the rainy season. As long as the weather is dry very few anophelines will breed in the Tropics, but the beginning of the rainy season is always marked by an immense increase in the number of these mosquitoes, and breeding places are easily found.

During hibernation the adult insects may be found in cellars, out-houses, stables, under the roofs of unceiled habitations, in dark closets, in hollow trees, or under shelving. In these situations they may be found in hundreds, or even thousands, and if disturbed appear sluggish and only fly for short distances.

I have already called attention to the fact that the ova of anophelines will withstand drying for some time, and I am of the opinion that in the Tropics many species would cease to exist were it not for this property, owing to the long dry season and the comparatively small number of hibernating females.

As regards the *number of generations* during a season our knowledge is very incomplete. According to the observations of Kulagin²⁵ the anophelines have only one generation annually. He calls attention to the fact that the female anopheline does not lay her eggs all at one time and states that, in temperate regions, the egg-laying extends throughout the entire summer, and that the adult mosquitoes derived from these eggs do not lay eggs until the following year. His observations extend over several years and are the most accurate that we possess upon this subject.

Nothing is known regarding this subject in respect to the mosquitoes of the Tropics.

Resting position of adults.—

The resting position of adult anophelines and the position assumed when biting, the two being practically identical, offers one of the easiest and most valuable methods of differentiating

between them and other mosquitoes. When resting upon a flat surface, as a wall, mosquitoes belonging to the *Anophelinae*, with few exceptions, form a distinct angle with the surface, the insect resting upon the first two pairs of legs, the last pair floating in the air or held out straight behind it. The angle formed by the body of the mosquito and the surface varies, but sometimes is nearly a right angle. The only exceptions to this rule among the anophelines commonly observed is *Myzomyia* (*Anopheles*) *culicifacies*, which rests upon a surface in the same position as a *Culex* mosquito, and *Pyretophorus* (*Anopheles*) *superpictus* and *Myzomyia* (*Anopheles*) *hispaniola*, which assume a perpendicular position in reference to the wall.

The position assumed by other mosquitoes, as *Culex*, *Stegomyia*, etc., is well described as "humpbacked," the abdomen approaching the resting surface while the thorax is distinctly higher than any other portion of the body, thus giving the humped appearance.

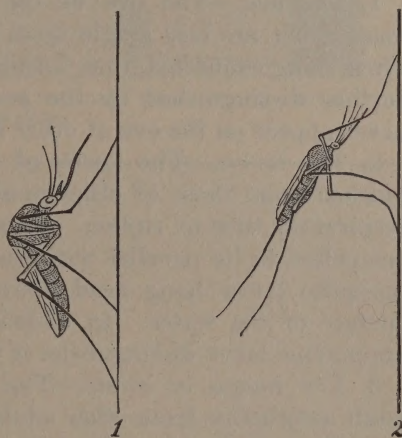


FIG. 3.—Comparison of resting position of *Anopheles* and *Culex*. 1, resting position of *Culex*, on vertical surface; 2, resting position of *Anopheles* on vertical surface. (The resting position upon a horizontal surface may be observed by turning the page so that the vertical line becomes horizontal.)

Whatever the position of a mosquito, it can always be determined whether it is an anopheline by attention to the relative position of the proboscis to the body. In the *Anophelinæ* the proboscis is always in a direct line with the rest of the body when the insect is resting upon a surface, while in all other mosquitoes the proboscis forms an angle with the abdomen. This peculiarity is easily recognized and gives the anopheline a distinctly "business-like" appearance, the entire body of the insect resembling a boring instrument.

Practical points in the differentiation of the Anophelinæ.—The following practical points may prove of use in the differentiation of anophelines from other mosquitoes in their various stages of development:

1. *The ova.*—The ova of the *Anophelinæ* are never found in masses, but are laid single upon the surface of the water, the ends often being connected, thus forming geometrical patterns. They are further distinguished by the possession of lateral "floats," which never appear on the ova of other mosquitoes.

2. *The larvæ.*—The larvæ of the *Anophelinæ* are easily distinguished from those of other mosquitoes by the absence of the long respiratory tube or siphon. Because of this the anopheline larva is compelled to lie parallel with the surface of the water, while other mosquito larvæ hang head downward at an acute angle with the surface of the water. In addition the small, narrow head of the anopheline larva distinguishes it from other mosquito larvæ.

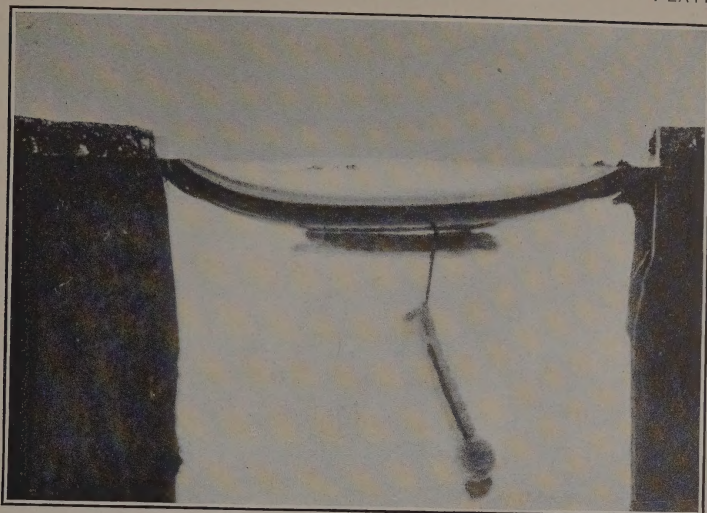
3. *The imago or adult.*—The following points distinguish the adult anopheline from other adult mosquitoes:

a. *The relative length of the palpi and the proboscis.*—In the *Anophelinæ* the palpi of the female are as long as the proboscis, while in other mosquitoes the palpi are much shorter than the proboscis. The males are distinguished from the females by their plumose palpi. By attention to this simple detail any mosquito may at once be placed so far as its relation to the *Anophelinæ* is concerned.

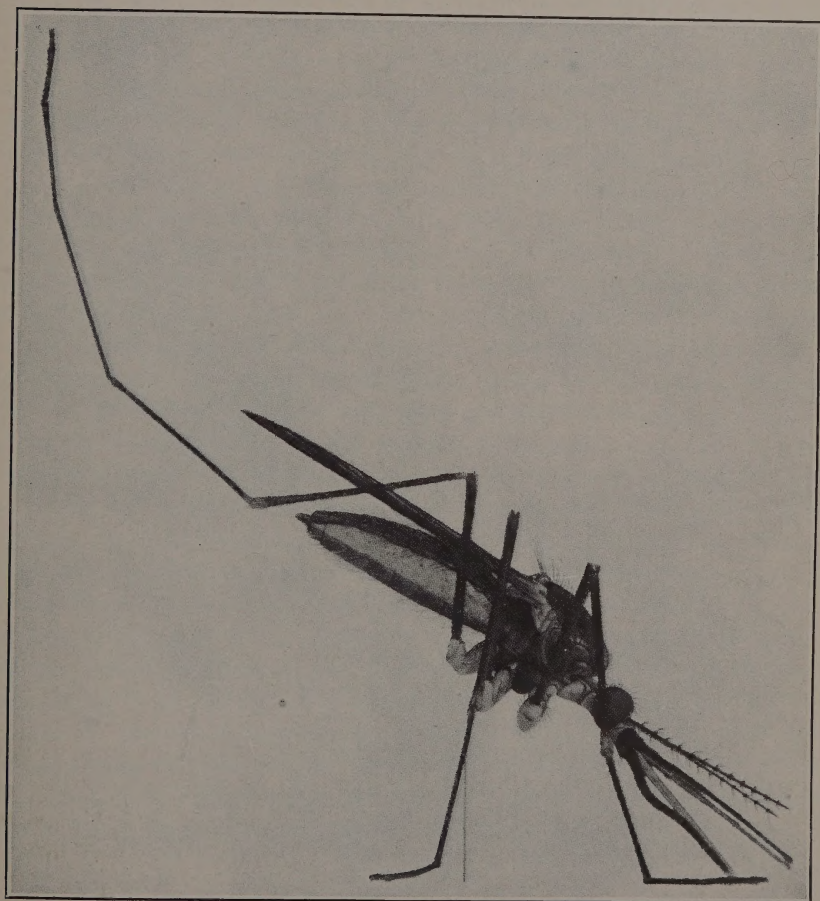
b. *The angle of the proboscis with the body.*—The proboscis in the anophelines never forms an angle with the rest of the body but continues in a direct line with it, as already stated.

c. *The resting position.*—When resting upon a surface most of the *Anophelinæ* form a distinct angle with the surface, while other mosquitoes appear humpbacked owing to the fact that the abdomen is nearer the surface than the thorax.

d. *The spotted wings.*—Most of the *Anophelinæ* have spotted wings and while some other mosquitoes also present spots upon these structures it is a good practical rule to always regard with suspicion any mosquito having spotted wings, as the chances are altogether in favor of its being an anopheline.



ANOPHELES AND CULEX LARVÆ IN NATURAL POSITION IN WATER.
(HOWARD.)



FEMALE ANOPHELES IN CHARACTERISTIC STINGING POSTURE.

Photograph of the model ($\times 75$) in the American Museum. Magnification of figure about 10 diameters.

METHODS OF COLLECTING AND DISSECTING MOSQUITOES.

The following directions for the collection and dissection of anophelines are all that are necessary for one engaged in practical prophylactic work. No attempt is made to describe methods of breeding the insects or of experimentation, as these phases of the subject relate more distinctly to the laboratory than to work in the field.

Collection.—The *ova* of the *Anophelinae* may be collected from the breeding places with a white agate ware dipper, but it should be remembered that they are very inconspicuous and have to be searched for very carefully.

The *larvæ* may be collected in a dipper from the breeding places by slightly muddying the water of puddles or shallow streams when they are more easily observed by reason of their grayish color. In small receptacles, water barrels, etc., they can easily be collected with the dipper. The *adult* insects are easily captured by the use of a collecting tube. At night they may be attracted by placing a lamp in such a position that a portion of the wall is but slightly lighted, when it will be found that the mosquitoes gather at the junction of the shaded area and can be easily captured. For this purpose a large test tube or a small straight glass cylinder may be used, the open end being placed over the mosquito as it rests upon the wall, then slightly moved across the surface of the wall, when the insect will fly to the closed end, whereupon the open end is removed quickly from the wall and plugged with cotton. If it is desired to kill the insects a small amount of cotton saturated with chloroform is placed at the bottom of the tube and covered with a disk of perforated cardboard, after which the tube is used as described. The utmost care should be used to avoid breaking the wings or otherwise injuring the insects if they are to be sent away for identification.

As the identification of the species of mosquitoes in any region may be a matter of great military importance, every effort should be made by medical officers to have the species identified in the localities in which they are serving. For this purpose the chloroform killing tube should be used to capture the mosquitoes, after which they are carefully slid from the tube into a small box containing absorbent cotton. Great care should be taken to avoid touching the insects or allowing the chloroform in the killing tube to come in contact with them. Not more than three or four mosquitoes should be placed in a box, and a label should be pasted upon the cover giving the place, date, and hour of collection, and any other data that is thought useful. If the box is to be mailed it should be inclosed in a larger box and packed carefully in cotton.

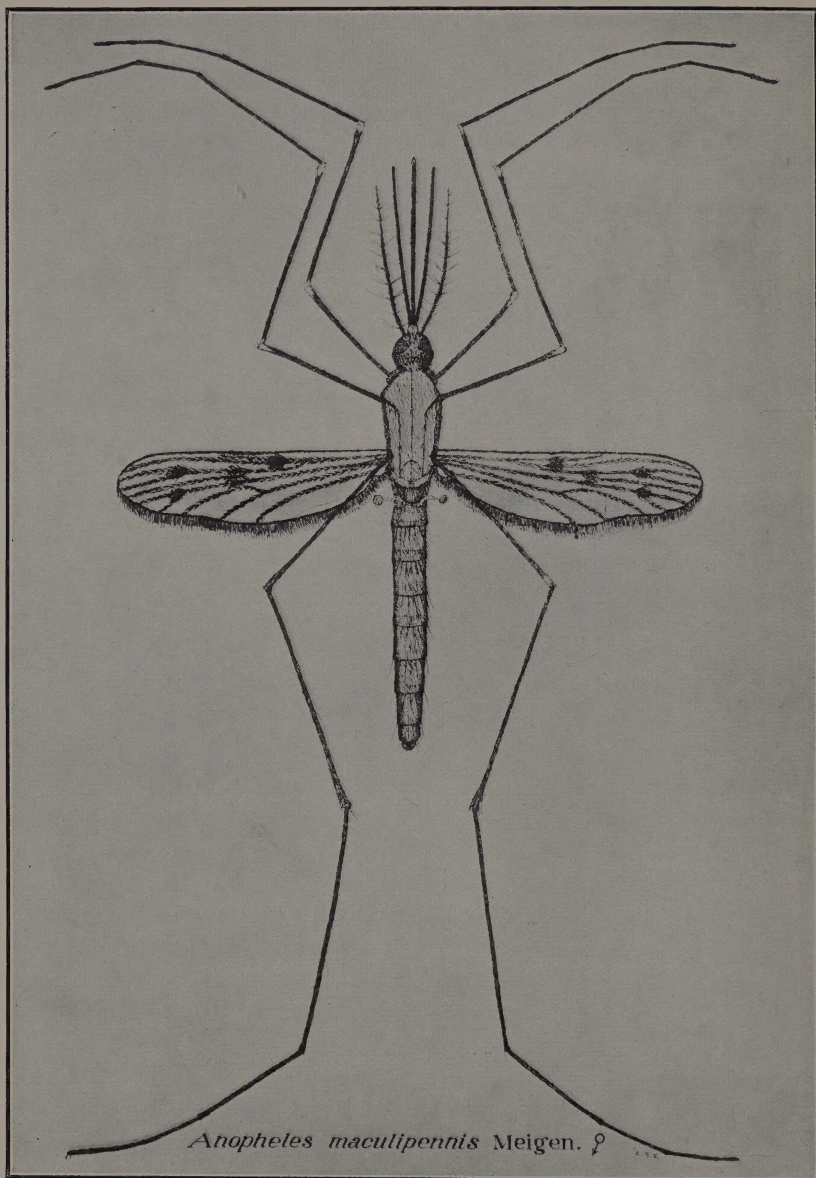
The dissection of mosquitoes.—To many the thought of dissecting a mosquito conjures up an amazing amount of difficulty, largely owing to the small size of the object to be dissected. As a matter of fact anyone possessing the most rudimentary knowledge of laboratory technique should have no trouble in dissecting these insects and demonstrating the presence or absence of malarial infection in them after having once been shown the really simple technique.

In order to make good dissections the following apparatus is necessary: Microscopic slides and cover glasses, fine dissecting needles, a good dissecting microscope, and some normal salt solution. A compound microscope, of course, is necessary for studying the development of the malaria plasmodia, but malarial infection of the mosquito may be demonstrated, in many instances, with the high powers of the dissecting microscope. The parts of the mosquito of special interest to one engaged in the prophylaxis of malaria are the salivary glands and the portion of the mid-gut of the mosquito known as the stomach.

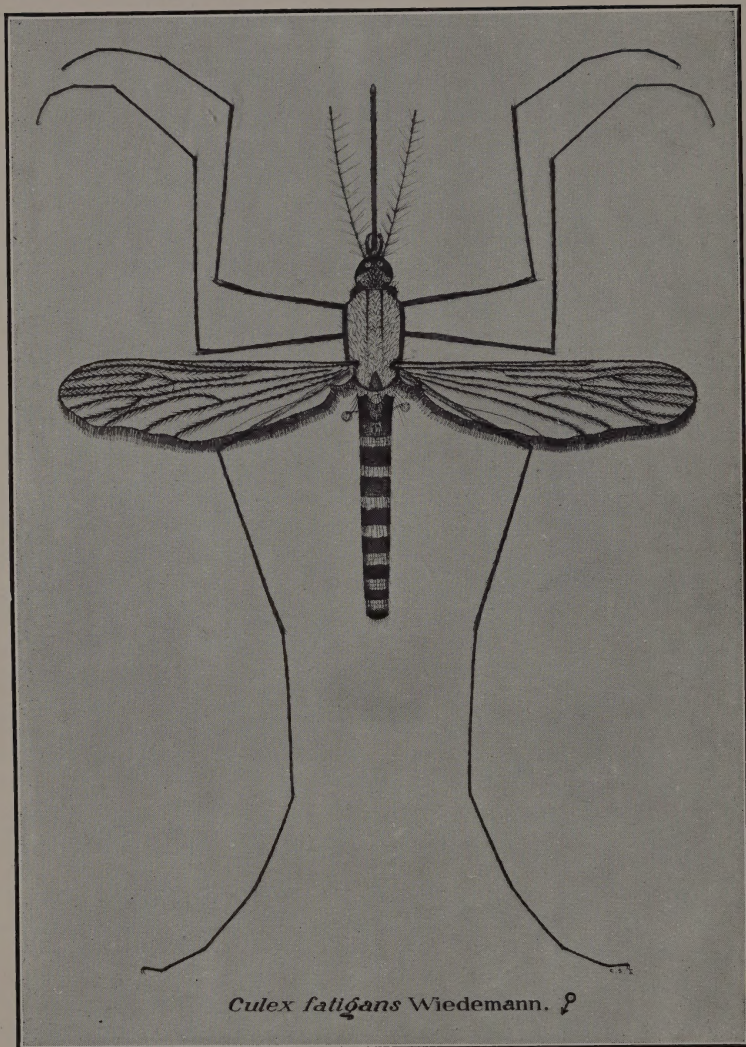
Dissection of the salivary glands.—To make a perfect dissection of the salivary glands of the mosquito requires considerable skill, but it is comparatively easy to secure portions of the glands sufficiently perfect to allow of demonstrating the *sporozoites*.

The salivary glands are situated within the thorax, lying ventrally, commencing at the posterior portion of the neck and ending at the level of the first pair of legs. The simplest way of securing them for examination is to cover the insect with salt solution and place it upon the stage of the dissecting microscope in such a position that the head is toward the dissector's hand. Then, with the needle in the left hand steadying the thorax, the chitinous covering of the thorax just back of the neck is incised, and then gentle traction is made with the needle in the right hand upon the head, separating it gently from the body. If the operation has been successful it will be noted that a minute bit of white tissue has been extracted with the head, and upon examination with the compound microscope it will be observed that in this bit of tissue lie the salivary glands, or portions of them. This tissue should now be removed from the head with a fine needle and placed upon a microscopic slide in a drop of normal saline.

In order to determine the presence of malaria *sporozoites* the preparation should be covered with a cover glass, gentle pressure applied, and examined with a one-sixth inch or one-twelfth inch objective. If *sporozoites* are present they will appear as fine, hyalin, curved rods, lying within the cells or ducts of the gland or free in the salt solution in great numbers. They often occur in masses or side by side and measure about 14 micros in length.



TYPICAL ANOPHELINE MOSQUITO.
Note long palpi and spotted wings. (Ludlow.)



TYPICAL CULICINE MOSQUITO.

Note short palpi and unspotted wings. (Ludlow.)

Dissection of the "stomach" of the mosquito.—To dissect that portion of the mid-gut of the mosquito, known as the "stomach," the following procedure is recommended:

The insect, from which the wings and legs have been removed by scissors, is moistened with a little alcohol or salt solution and then placed back downward upon the glass stage of the dissecting microscope or upon a glass slide placed upon the stage. Using a needle in the left hand to steady the insect, a nick is made in the chitin of the thorax on either side as near the posterior end as possible; the left needle is now placed firmly upon the thorax and the right upon the last abdominal segment, and gentle traction exerted by the right hand, when between the separating segments a white mass of viscera is observed which contains the mid-gut, hind-gut, Malpighian tubules, and ovaries, or may contain only a portion of these. The mid-gut is separated, and will be found to consist of an anterior tubular portion and a posterior sacular portion, which is the so-called "stomach" of the mosquito and which contains the malarial cysts or *zygotes* if they be present. This portion of the mid-gut is placed upon a slide and examined with a one-sixth lens; but if it contains blood it should be discarded, as in this case the *zygotes* are apt to be overlooked.

If the fully developed cysts are present, they may be seen with the one-sixth lens projecting from the wall of the stomach or apparently embedded in the wall. They may often be picked up quickly by attention to the presence of pigment, for some of them contain a small amount of melanin. The earliest stage observed is an oval or spherical body about 8 microns in diameter and containing more or less pigment, which may or may not be motile. As the *zygotes* increase in size, a well-defined capsule or cyst wall develops, and when the *sporozoites* have developed they may be seen packing the cysts, which have become so large that they may be observed with a one-third-inch lens. The pigment decreases in amount as the *zygotes* develop, and when they are fully developed many of them are entirely devoid of pigment.

For a more minute description of the technique of the examination of mosquitoes and of the morphology of the malaria plasmodia within the mosquito, the reader is referred to any of the larger works upon the malarial fevers which are given in the list of references in this bulletin.

CHAPTER III.

PROPHYLACTIC METHODS BASED UPON THE DESTRUCTION OF MALARIA MOSQUITOES.

In the prophylaxis of the malarial infections the following measures have been found efficient in practice: 1. The destruction of the mosquitoes transmitting the infection; 2. The protection of man from the bite of mosquitoes; 3. The destruction of the malaria plasmodia while in the blood of man. Theoretically, any one of these methods would be sufficient to prevent malarial disease could it be applied in an ideal manner, but practically, it is but seldom that one is successful without the aid of the others, and it is generally necessary to combine them all in order to achieve the fullest success in the prevention of malaria in any locality.

In this chapter will be considered those methods of malaria prophylaxis that depend for their success upon the destruction of the mosquitoes transmitting the disease, and in applying these methods it is necessary that one have knowledge of the life history of the *Anopheles* to the extent comprised in the data given in the preceding chapter. It is obvious that before applying most of the methods mentioned in this chapter it will be necessary to make a mosquito survey of the locality one is working in, thus determining whether malaria mosquitoes are present or not, their breeding places, and the preventive methods most applicable and economical for the region investigated.

The prophylaxis of malaria by the destruction of mosquitoes is the *ideal method of prophylaxis* where conditions are such as to render the destruction of these insects practicable, but in the military service such methods must necessarily be restricted to more or less permanent camps or to permanent posts, as most of them are impracticable upon the march or in camps of short duration. In many regions in the United States where military posts are located it is believed that the malaria present could be entirely eradicated by the adoption of methods looking to the destruction of mosquitoes, and this may also be true of isolated instances in our tropical possessions, but in most regions, especially in the Tropics, the most that we can hope to accomplish in the way of mosquito destruction is to greatly reduce the number of the insects, and in such localities other methods of prophylaxis must be used in conjunction with the destruction of the mosquito.

The malaria mosquitoes may be destroyed at any stage in their development, but most of the prophylactic methods depend for success upon the destruction of the insect during the larval and adult stage, the larvæ being destroyed by the abolition of the collections of water in which they have developed or by larvacides, and the adult insects by various chemical agents or other measures. The most important measures consist in the abolition of the breeding places of the mosquitoes by leveling, drainage, and clearing, and if they can be carried out in an efficient manner they are the most valuable that we possess, and in the vast majority of instances are capable of greatly reducing, if not almost eradicating, malarial disease. Unfortunately, the terrain often renders the application of these measures very expensive and other prophylactic methods are substituted, but, where it is possible, I believe that these measures, although the first cost may be great, should be the ones adopted, as they produce permanent results and in the case of many of our Army posts would banish malaria from the sick report.

Drainage.—If possible all large breeding places of mosquitoes should be drained, if they can not be filled in or otherwise abolished. If filling in is feasible, this is the better method, but the relative cost of the two methods must be considered for each locality and the choice made as to which will be the cheaper and most efficient.

If drainage is decided upon, the question arises as to the particular kind of drainage to be used. In many temperate and subtropical regions open ditches may be used, provided the edges are kept free from plants and algæ and there be enough slope to give a comparatively swift current. Otherwise, the open ditch, although it may drain a larger body of water, simply becomes a mosquito breeding, and this is almost sure to happen in the Tropics, where it is practically impossible to prevent the growth of plants and algæ in the water of the ditch. This fact has been well brought out by Le Prince²⁶, who says:

On the Isthmus and probably throughout the Tropics, under average conditions, we could not consider such a mode of procedure (the open drainage ditch) as it would give most unsatisfactory results. Such a ditch would become a veritable Mecca for anopheles.

In order to prevent the development of vegetation in the ditch it would have to be cleaned out in the Tropics on an average of once a week, and this alone would make their use practically impossible, as the cost of labor and upkeep would be too great unless the ditches were few in number and of limited extent.

The blind drain.—Le Prince,²⁷ the chief sanitary inspector of the Isthmian Commission in 1908, recommends this method of drainage in case tile drains can not be obtained. He defines a blind drain "as a miniature culvert covered with field stones," and states that

they give fairly good results. Such drains are constructed as follows: A ditch is dug and the bottom covered with flat stones, the sides are formed by a layer of flat stones, and a cover stone is placed on top, after which the whole is covered with quite large stones and lastly with small stones. Regarding this type of drain Le Prince says:

Where plenty of stone is available and must be removed from a field, this form of ditch is economical to make and, in the Tropics, much more economical to maintain than an open ditch is, while anopheles larvæ do not occur therein * * *. We opened some of these drains when the flow of water in them was sluggish, but found no mosquito larvæ therein. The advantage of this type of drain is that there is no first cost for material, the field stones are disposed of, and there is no cost of maintenance except when the ditch clogs up. A good grade is essential for blind drains, and they will stand on a fairly heavy grade.

The cement-lined open drain.—This is the only form of open drain that should be adopted in the Tropics, and it should always be laid upon a considerable grade. Such drains should be semicircular in shape and constructed of stone or brick, lined with cement. If laid upon a good grade such drains will keep themselves clean by the flushing of the water and will not become filled with vegetation and algæ.

Subsoil drainage with tiles.—Le Prince says that “the use of tile drainage is the most economical and permanent method of destroying anopheles in the Tropics. The work costs from 16 to 20 cents a foot upward, according to the depth of the trench, material to be excavated, and distance that the tile and covering stone has to be carried.” He recommends that the tile drains should not be given a flatter grade than 0.5 per cent, and states that those having as high a grade as 5 per cent gave perfect satisfaction if the tiles be covered with plenty of stone to hold them in place. This is the method of drainage adopted in the Canal Zone, where it has proven less expensive in the end than the open ditch and much more efficient.

The question of the prevention of mosquitoes by drainage is a purely economical one, as there is no question of its efficiency in any region where it can be applied. In the Tropics its application is much more difficult than in temperate regions, but the success of this prophylactic method in the Canal Zone proves that even in the Tropics it can be used under the most difficult conditions and that its use is both economical and successful. In many of our posts a system of tile drainage would completely eradicate mosquitoes at a much less final cost than is now expended in screening and for mosquito nets.

Filling.—In many localities the filling in of low ground and of small pools will serve to destroy most of the breeding places of mosquitoes, and this is often a very valuable prophylactic method.

Removal of shelter.—In conjunction with drainage and filling in of the breeding places of mosquitoes, the removal of brush along

the banks of streams and of vegetation and algæ in the streams or of any shelter for the mosquitoes, such as is furnished by jungle, long grass about residences, and vines and bushes, is a valuable prophylactic measure. The removal of the vines so often observed inclosing the porches of barracks and quarters in some of our most malarious posts should be insisted upon if mosquitoes can not be eradicated, as they furnish shelter to many of these insects, and their removal will result in a great diminution in the number of mosquitoes and, consequently, in the prevalence of malaria. The same is true of all shrubbery about habitations, and the measure adopted in the Canal Zone of removing all such vegetation for 200 yards around the houses could be followed with excellent results in more than one of our Army posts.

The clearing of rank vegetation and jungle for several hundred yards around a camp or post is not only useful because it deprives the mosquito of shelter, but it also results in the discovery of the small breeding places of the mosquitoes which could not otherwise be seen. The anopheline mosquito does not require a lake to breed in but, as has already been stated, will breed in the smallest depressions in the ground that will hold water, and every clearing operation, especially in the tropics, always results in the discovery of many of these minute breeding places.

At Camp Stotsenburg, in the Philippines, it was invariably noted that mosquitoes increased greatly in number whenever the grass about the post was allowed to grow to any great length and that a coincident increase occurred in the number of malarial cases admitted to hospital. At one time, owing to lack of facilities for cutting, the grass was allowed to grow for several weeks, and the mosquitoes became so numerous as to be almost unbearable, while the number of cases of malaria admitted to the hospital became alarming. As soon as the grass was mowed the mosquitoes decreased in number, as did the number of malarial infections, thus conclusively proving the danger of affording shelter to the mosquito.

Destruction of mosquito larvæ with larvacides.—While the drainage or filling in of the larger bodies of water that are breeding places for mosquitoes is the ideal method of dealing with them, it frequently happens that this is impossible owing to various local conditions. When this is the case the larvæ may be killed by certain substances which may be added to the water, and this furnishes us with a prophylactic method of considerable value.

Many substances have been recommended for this purpose, the most important of which will now be considered:

Petroleum.—The use of kerosene as a larvacide was first recommended by L. O. Howard,²⁸ and it has been used very largely by the

Army, both in this country and in Cuba and the Philippines, with excellent results. This method of prophylaxis depends for success upon the fact that the larvæ of mosquitoes are unable to breathe if the surface of the water is covered with a film of oil, and it therefore follows, that in selecting petroleum for this purpose an oil should be chosen that will spread rapidly when placed upon the surface of water and one that will not evaporate too rapidly. Howard recommended to the Army in Cuba the use of the grade of oil known as light fuel oil, costing from \$2.25 to \$3 per barrel, and this was used with good results. A thicker oil, composed of 4 parts of oil of 18° gravity and 1 part of oil of 34° gravity, is recommended by Quale, who used it in California and found that it remained efficient for from three to four weeks after application.

The application of the oil, in the case of large bodies of water, is best made with a pump having a straight nozzle. The operator is rowed from side to side of the pond and the oil discharged at such intervals that when it rises and spreads the entire surface passed over is covered. In smaller collections of water an ordinary watering pot may be used with a spray nozzle or the oil may be poured from a cup or dipper. The greatest care should be taken to see that there are no portions of the surface left uncovered after the oil has spread, and all oiled collections of water should be inspected at least every third or fourth day, as the layer of oil becomes easily displaced by currents in the water, by winds, or by the movements of aquatic animals. Whenever unoiled areas appear upon the surface the oiling of that particular portion of the pond should be repeated. In the case of more or less stagnant streams or ditches the oil is best applied with a pump and the application frequently repeated. The pumps used for this purpose are the ordinary knapsack pump or the bucket pump.

The exact time that the oil will have to be renewed varies greatly, and in many instances the method is rendered almost useless by ignorance regarding the necessity of renewing the oil at frequent intervals. While, in rare instances, one application of oil may be efficacious for as long a period as four weeks, in the vast majority of cases the application will have to be renewed much within that time. Where the temperature is high and the surface of the water is exposed to the sun, the oil evaporates rather rapidly and has to be renewed at much shorter intervals than where the temperature is low and the collection of water is well shaded.

It would seem that the most sensible rule for renewing the oil would be to reoil every two weeks. We know that the average life of the larvæ of mosquitoes is from 10 to 14 days, and if the oil be reapplied at these intervals we will be sure to destroy the vast majority of the larvæ. However, if inspection of the oiled water

shows that the oil has in part or wholly disappeared before the end of two weeks, it is obvious that reoiling should be done at once. The two-week interval was the time adopted by the Army medical officers in Cuba and was found to give satisfaction.

In some regions the use of oil as a larvacide is practically impossible owing to the currents produced by the strong winds of the locality or to the development of tropical vegetation in the collections of water to be oiled, thus preventing the spreading of the oil over the water surface. In such instances the use of a larvacide that is less easily displaced by winds and that is capable, to some extent, of killing vegetation is indicated, but up to the present time such an ideal larvacide has not been discovered, the nearest approach to it being the one used in the Canal Zone and which will now be described:

The Canal Zone larvacide.—This preparation has been very largely used in the Canal Zone, and all of the reports concerning its action are very satisfactory. Darling²⁹ has described the method of manufacture and has conducted valuable experiments determining its efficiency when brought in contact with the larvæ of both *Culex* and *Anopheles* mosquitoes. He thus describes the method of preparing the larvacide:

One hundred fifty gallons of crude carbolic acid (specific gravity not greater than 0.97 and to contain not less than 30 per cent tar acids) are heated in an iron tank having a steam coil with steam at 50 pounds pressure. Two hundred pounds of finely crushed and sifted common rosin are dissolved in the heated acid, and then 30 pounds of caustic soda, dissolved in 6 gallons of water, are added. There is a mechanical stirring rod attached to the tank. The product is ready in a few minutes, yielding about $3\frac{1}{2}$ barrels.

Regarding the value of this preparation as a larvacide, he says:

The resultant emulsion makes a very good disinfectant or larvacide. In fact, 1 part of it to 10,000 parts of water will kill anopheles larvæ in less than half an hour, and 1 part to 5,000 parts of water will kill anopheles larvæ in from 5 to 10 minutes or less. This property of killing larvæ rapidly is of great importance in the Tropics, where continuous rainy periods make crude oil or kerosene much less valuable as a larvacide than it is in northern latitudes having less rainfall. Also the larvacide acts as an algicide, and thus destroys the food and hiding places of anopheles larvæ.

This larvacide is used by spraying an emulsion, prepared by adding 1 part of the larvacide to 5 parts of water, over the surface and along the edges of ponds, streams, or other collections of water that serve as breeding places for mosquitoes.

Used in this manner the larvacide will kill all anopheles larvæ within five minutes, and it possesses the advantage that after a few hours, in case of running streams, the water will be again fit for domestic use.

This larvacide would appear to be an ideal one for adoption by the Army in its antimosquito work, especially in the Tropics where

the use of kerosene is expensive and often inefficient. The cost of preparing it is stated by Darling to have been but \$0.1413 per gallon, which is very much less than any fuel oil can be purchased for, and 1 gallon of the larvacide makes 6 gallons of the preparation when diluted with water for use in the spray. In addition this larvacide is a good germicide, having a Rideal-Walker coefficient of from 2 to 5. One, or at most two, larvacide plants could supply all the larvacide needed in the Philippines, and the same is true of the United States, and the manufacture and use of this substance by the Government would result in a considerable reduction in the cost of malaria prophylaxis and at the same time furnish the Army with a more efficient larvacide than kerosene oil.

Other larvacides.—Among other substances that have been recommended as larvacides may be mentioned permanganate of potassium, sulphate of copper, corn oil, Phinotas oil, and many proprietary preparations. None of these are as efficient as the larvacides already mentioned, and the only one that has been used extensively is Phinotas oil, which has been used to a considerable extent upon the Isthmus of Panama. It is capable of quickly killing the larvæ, but also kills fish, so that it should not be used where it is desired to preserve the latter.

Although it is possible to secure very excellent results in the prophylaxis of malaria by the use of larvacides the method is always an admission that we are unable to control mosquito breeding by the much better one of abolishing the breeding places of these insects, and it should never be adopted in preference to the latter. Under the best conditions it is imperfect and the results are not to be compared with those obtained by drainage or filling in of breeding places. In addition, especially in the Tropics, the method is more expensive, because the results are not permanent, the use of the oil having to be continued for months, while, even in temperate regions, it is doubtful if this method is as economical as the abolition of the breeding places. It finds its greatest field of usefulness in those instances where breeding places can not be abolished owing to the expense involved, or where open ditches and streams can not otherwise be prevented from becoming breeding places of these insects.

Destruction of larvæ by fish.—The well-known fact that several species of fish are voracious devourers of mosquito larvæ has been taken advantage of in the prophylaxis of malaria, and there are many accounts in the literature of attempts to render the breeding places of mosquitos harmless by the introduction of fish that will devour the larvæ. There is no question that in many instances these attempts have met with a considerable amount of success but, as a rule, the results have been disappointing. The fish that are the most useful are the roach, carp, top minnows, and the goldfish, the roach

and top minnows especially being very active in devouring the larvæ. Where there are small ponds or artificial collections of water of limited extent the stocking of them with these fish will often result in keeping them comparatively free from mosquito larvæ, but the method, at best, is a very imperfect one, and possesses little of value for the military sanitarian, although, in very exceptional cases, it might yield good results.

The abolition of breeding places in and about quarters and barracks.—It has already been stated that anopheline mosquitoes will breed in even the smallest collections of water and that, with several species, it makes no difference what the quality of the water may be. The old idea that the *Anopheles* will only breed in clean water and only in streams or larger collections of water has long ago been abandoned, and we now know that they will breed both within and without quarters and barracks wherever a collection of water forms large enough to contain the mosquito eggs. This renders the sanitation of quarters and barracks of the very greatest importance in any campaign against malaria in a military post, and it is remarkable how many breeding places may be discovered around and in quarters and barracks that are believed to be perfectly free from them. Among the breeding places that occur in the quarters and barracks may be mentioned uncovered fire buckets, stopped drains, the unused hoppers in toilets, unscreened water tanks, earthenware vessels containing water, tubs, water traps that are not flushed out frequently, and the cans of water that are often used in the Tropics to place table legs in to prevent ants from getting to the food. All of these are favorite breeding places of certain species of *Anopheles* and are very frequently overlooked by those having little knowledge of the habits of these mosquitoes.

If the post is in the neighborhood of native quarters, as generally happens in the Philippines and our other tropical possessions, the condition of these quarters as regards the breeding of mosquitoes becomes of paramount importance and necessitates the greatest care in inspecting and abolishing the numerous breeding places that are always present around and in native houses. An inspection of the premises will generally reveal larvæ in the water that has collected in broken bottles, old culinary utensils, tin cans, tin-lined boxes, water barrels, and chicken troughs. In addition, puddles of water will often be found teeming with larva in the immediate vicinity of the dwellings, and even the bamboo fence that surrounds so many native houses will be found to breed numberless mosquitoes in the cavities left by cutting off the bamboo near a joint or where the mosquitoes have gained entrance through holes made by boring insects.

The discovery of these small breeding places of mosquitoes and their proper treatment forms one of the most important duties of sanitary officers in localities where malaria is endemic, and the ability to make a really scientific mosquito survey of a post and its surroundings should be considered one of the most valuable of all possessions to one who is engaged in the prevention of this disease.

All receptacles in which mosquitoes may breed and which are not needed in domestic operations should be removed or destroyed, and those in which it is necessary to keep water should either be frequently emptied, screened, or oiled. Barrels or tubs for the collection of rain water, as well as cisterns, should be covered with well-fitted screen covers, made of wire containing not less than 18 meshes to the inch. Unused hoppers of toilets and water traps should be flushed thoroughly at least once a week, and drains should be frequently inspected and flushed. One of the most common sources of mosquitoes connected with barracks and quarters are clogged roof gutters, and I have frequently found such a gutter alive with larvæ after heavy rains that had left the gutter full, followed by enough light rain to prevent total evaporation of the water. The inspection of all roof gutters and their repair, where clogged, is an important prophylactic measure in Army posts.

The fire buckets found in most barracks are prolific sources of mosquitoes unless they are properly looked after. All that is necessary in order to prevent the development of larvæ is to see that they are emptied each week and filled with fresh water. As the average life of the larvæ is about 14 days, one is perfectly safe if the buckets are emptied each week. If desired, wire screen covers may be used, but these are expensive and unnecessary if the buckets be properly emptied and refilled at weekly intervals. The oiling of the water in fire buckets, so frequently observed, is an evidence of the lack of knowledge of the life history of mosquitoes on the part of the officer ordering such a procedure, and certainly the addition of oil to water destined to extinguish fire does not improve its value in that direction. The buckets should not be emptied into the sewer, but upon the ground in direct sunlight where the water will quickly evaporate and the sun kill any larvæ that may be present.

Water troughs for domestic animals should be flushed frequently, and in no case should the water be allowed to remain if the trough is not in use. The tanks in water-closets, the sewer traps of the post, and open cesspools, if present, should be frequently inspected and properly treated, the tanks and sewer traps by flushing or oiling and the cesspools by oiling at intervals of two weeks.

The best way to accomplish the abolition of the small breeding places mentioned is to place the work under the charge of a sanitary officer, who should be assisted by a good sergeant and the

requisite number of men. If the post is a small one the work can be easily accomplished by a sergeant and one or two men, but if the area to be covered is large and the buildings are numerous the post should be divided into districts, each district being looked after by one man, under the supervision of the sanitary officer and the sergeant. Where native barrios or villages have to be sanitated it will be necessary, of course, to increase the number of men, but the same general plan of organization should be followed.

Destruction of the adult mosquito.—The adult mosquito may be destroyed by various chemicals or they may be caught in mosquito traps or by hand. In the military service the methods of destroying adult mosquitoes that can be adopted are in general too uncertain to be of much value except in fumigating quarters that are to be occupied by troops in countries where malaria is endemic or upon transports plying between malarial and nonmalarial localities. In the field the adult mosquito is most easily avoided by methods which prevent biting and which are much more practical in application than attempts to destroy the insects, although smudges, hereafter described, may prove useful in isolated instances.

A large number of chemicals have been recommended for the destruction of adult anophelines, the most important of which will be briefly discussed.

Sulphur dioxide.—Where it is desired to kill the mosquitoes in quarters or barracks, in transports or other vessels used for military purposes, or in habitations in malarial localities, sulphur dioxide is the most valuable agent that we possess. The success attending its use in Cuba, Panama, and in Rio de Janeiro in the fumigation of houses, and, indeed, whole blocks of houses, as at Santiago, against the yellow-fever mosquito, is familiar to all sanitarians, and the same methods used against yellow fever should be employed in the case of malaria where the disease is of pernicious type and rapidly spreading among troops in quarters.

In employing this agent it should be remembered that it injures most metallic substances and fabrics, and these should be removed before fumigation is begun. The methods of fumigation with sulphur are well known to all sanitary officers of the Army and will not be detailed here. A good rule for determining the amount of sulphur necessary to destroy the mosquitoes in fumigation upon a large scale is to divide the number of cubic feet in each room of the building by 500 and reading the result in pounds. Thus, a room 40 feet long, 20 feet wide, and 12 feet high would contain 9,600 cubic feet, and this divided by 500 would give 19.2 pounds of sulphur for such a room.

In the case of malaria it is evident that only in rare instances would this method of prophylaxis be used in the military service,

owing to the fact that other and more easily applied methods would render it unnecessary. However, there is no doubt of its great value in the prevention of the transmission of yellow fever and in great epidemics of malaria, such as have occurred among troops in quarters, it would undoubtedly be of equal value.

Smudges.—In the field many adult mosquitoes could be destroyed by fires made of material producing a dense smoke, as green vegetation or bark. The American Indian used this method of rendering his tent inhabitable and all hunters are familiar with this device, which might prove very useful to an army in the field in regions where mosquitoes were very numerous and mosquito nets unobtainable.

Pyrethrum powder.—Pyrethrum powder is made from the dried flower heads of plants belonging to the genus *Chrysanthemum*, growing in Transcaucasia. The powder is more commonly known as Persian or Dalmatian insect powder, and depends for its efficiency on the presence of certain oleoresins in the dried flowers, so that to be efficient the powder must be comparatively fresh. The best preparation for use in this country is made in California and is marketed under the proprietary name of "buhach" powder.

For the purpose of destroying mosquitoes the powder is burned in a room, being placed upon a dish or tin and a match touched to it, when it will burn slowly and give off a large volume of smoke having a peculiar odor; or the powder may be procured in the form of cones or pastilles and these burned. The smoke is not harmful or very unpleasant to most people, and if the powder be burned in a closed room it will stupify all mosquitoes in the room and they may be swept from the floor and destroyed. The use of this powder in the rooms of quarters infested with mosquitoes might be of benefit where other measures could not be taken to get rid of them, but from a military standpoint this method of destroying mosquitoes possesses little value.

Numerous other fumigants have been recommended for destroying adult mosquitoes, as camphor phenol or Mimm's Culicide, Pyrofume, mercuric chloride, powdered stramonium, and formaldehyde gas, but none of them are of any importance in the prophylaxis of malaria in the military service and will not be discussed.

Mosquito catching as a prophylactic method.—The catching of adult mosquitoes, first advocated by Orenstein,³⁰ in the Canal Zone, is really deserving of much greater consideration as a prophylactic method than it has received. It is a method that could readily be adopted in the military service and, under certain conditions, would be a most valuable one, especially in temporary camps in the Tropics. In his paper, published in 1913, Orenstein states that this method has been extensively employed in the Panama Canal Zone and that

it has given very excellent results. He quotes two instances given by Le Prince, which are so striking that I will give them in his own words. He says:

The first systematic application of mosquito catching within habitations as a prophylactic measure against malaria, in so far as I have been able to learn, was made in the Canal Zone, in April, 1908, at a camp near Cocoli. The camp, composed of unscreened tents, was to be maintained only about five months, and it was not thought practicable to incur the great expenditure involved in a thorough antimalarial campaign. When the camp was first established, as many as 30 anophelines could be seen in a tent in the daytime, and many more after dark. One man was employed to kill the mosquitoes in these tents. He worked from 6 to 11 in the morning and from 1 to 5 in the afternoon. In the four and a half months of this camp's existence the malaria incidence was a little over 1 per cent per week.

Another remarkable instance is described by Le Prince in Ross's "The Prevention of Malaria." In June, 1908, several hundred United States marines were quartered for about eight weeks on a hill near Panama, known as Diablo Hill. During this period the malaria incidence among the marines was 14 per cent per week. In May, 1909, some living cars were located on the same hill. The anopheline breeding was just as it has been at the time the marines camped there. From the first week in May to the end of November, in the rainy season, when malaria incidence is high in Panama, only four cases of malaria occurred among the 40 laborers occupying these cars, a weekly incidence of about 0.3 per cent. The difference was due to having a man devote half an hour a day to the destruction of the anophelines found in these cars at a cost of 5 cents a day for the labor."

The above illustrations demonstrate that mosquito catching is capable of keeping the malaria incidence down to a very low figure even in hotbeds of the disease, and indicates how valuable a method this would be in the military service, where it could so easily be adopted.

The mosquitoes are caught with the chloroform tube already described in Chapter II, although Orenstein recommends that instead of cotton at the bottom of the tube to receive the chloroform a layer of short lengths of rubber bands covered with a plug of cotton be placed at the bottom of the tube and saturated with chloroform. In addition to the killing tube the mosquito hunter is also furnished with an ordinary "fly swatter" and instructed to catch or "swat" every mosquito observed. The method of catching mosquitoes with the tube has already been described (Chapter II, p. —), and the mosquitoes should be searched for in dark corners of rooms, in closets, along baseboards, beneath sinks and toilet fixtures, behind pictures, and in tents, along the ridgepole, in the corners behind clothing, and beneath cots and mosquito nets. The "fly swatter" is especially useful in killing the mosquitoes frequently observed on the outside and inside of screenings on verandas, doors, and windows. At Camp Stotsenberg, during the rainy season, the outside of the window screens of the hospital and other buildings was frequently the resting

place of scores and even hundreds of anophelines which were endeavoring to get within the buildings, and it was possible to kill hundreds of these insects in this situation almost every evening during the rainy season.

Mosquito traps have also been used extensively in the Canal Zone, and Orenstein describes one devised by Sanitary Inspector Bath, which has proved satisfactory. It consists of a half-cylinder constructed of wire netting, having two ridges inside it, "the apices of which are perforated by longitudinal slits about $\frac{1}{4}$ inch wide and 3 inches long. Through the slits in these ridges the mosquitoes enter the chamber of the trap, and can not, for some reason, find their way out again."

The traps are so placed as to catch the mosquitoes either when entering or leaving a building, and in the Canal Zone it was found that more anophelines were caught if the traps were placed on the lee of buildings, while more culicines were captured if the traps were installed on the windward side of the buildings.

Regarding the value of catching mosquitoes Orenstein well says that:

When it is remembered that in malaria a period of at least a week must elapse before the mosquito which has fed on an infected person can transmit the infection to another person, and when it is recalled that an anopheline filled with blood becomes sluggish and does not fly very far for some time, the efficacy of killing mosquitoes within habitations becomes self-evident.

And he concludes that "catching mosquitoes by hand within dwellings is a measure of great value in the prophylaxis of malaria. It is especially applicable to temporary camps."

It is obvious that this prophylactic method might be of the very greatest value to the military service in the case of camps in malarial localities and it could be so easily put in operation that its neglect would be inexcusable. All that would be necessary would be to detail a certain number of men for the purpose, under an intelligent non-commissioned officer, the camp being districted for the purpose. No great amount of training is necessary, as mosquitoes are easily recognized and the method of handling the killing tube and "swatter" is quickly acquired even by the most unintelligent laborer, as shown by the fact that all the work in this line in the Canal Zone was done by the West Indian negro. In the field where troops are in tents for some time, as at maneuvers or in more or less permanent camps, and where mosquitoes are prevalent, this method is certainly deserving of the most thorough trial.

In barracks and quarters in posts situated in the Tropics mosquito catching would also be a valuable method of prophylaxis and could be accomplished by native help at a comparatively small expense. In many posts in the Philippines, where it has been found impossible

to entirely eradicate mosquitoes, this method would be easy of application and would undoubtedly give good results in reducing the number of mosquitoes and the incidence of malaria. In temperate regions it is generally possible to rid our posts of mosquitoes by one or more of the other methods described in this chapter, but in the Tropics it is very often impossible to do more than reduce the number of these insects, and this would appear to be a great aid in that direction, and possesses the additional value that it enables us to capture and kill the mosquitoes that have bitten man and may, therefore, have become infected with malaria.

CHAPTER IV.

PROPHYLACTIC METHODS BASED UPON THE PROTECTION OF MAN FROM THE BITES OF MOSQUITOES.

Where it is impossible to destroy all of the breeding places of mosquitoes or to kill the adult insects, the protection of man from the bites of these insects becomes a most important prophylactic measure. Especially has this been found true in the military service, where this method, together with quinine prophylaxis, often has to be depended upon entirely when troops are in the field in malarial localities, and even in semipermanent camps and permanent posts the protection of the troops from the bites of mosquitoes not infrequently becomes of paramount importance owing to local conditions or for economical reasons. While, as has been stated, the abolition of the breeding places of mosquitoes is the ideal method of malaria prophylaxis either in military or civil communities and other methods should never be substituted for this where it is possible to employ it, the fact remains that in most localities it is necessary to combine all of our methods of prophylaxis, and of them the protection of man from mosquito bites is one of the most valuable.

This mechanical prophylaxis, as it is sometimes called, is secured by the proper screening of quarters and barracks, the use of mosquito nets for the beds and shelter tents, the wearing of head nets and gloves, and the use of various odorous substances which are smeared upon the skin for the purpose of preventing mosquitoes from biting.

Screening.—The screening of barracks and quarters in posts in malarial regions is a most important and valuable prophylactic measure and should never be neglected when the more permanent methods of drainage and filling in can not be employed or where their employment only reduces the number of anophelines. While, in civil life, the question of expense makes the employment of this method prohibitive in many localities, in the military service it should not enter into the question where posts are situated in regions where pernicious forms of malaria are prevalent, or where any form of malaria is prevalent to an extent that will seriously affect the efficiency of troops.

The expense involved is admittedly great, in many instances, but if good screening material be obtained, the screens will, if properly cared for, last for years, and before they are worn out will have repaid many times the initial expense by the reduction in the amount of malaria among the troops and the consequent increase in efficiency.

The selection of screening material.—The screening used for quarters and barracks should be of as durable material as it is possible to secure and of fine enough mesh to keep out all anophelines. The *material* for screening should be of wire composed of copper, zinc, and iron, the copper content being higher than that of brass. In the Tropics this is of special importance, as the heat and moisture rapidly corrode wire netting having a low percentage of copper. The observations of Nauss, in the Canal Zone, reported by Darling,³¹ proved that the deterioration of wire screening in the Tropics is due to the presence of iron plus the influence of a hot and moist climate. Brass screens, by reason of the amount of iron alloy present are, therefore, unsuitable, and copper-wire screens should always be selected.

If copper screening can not be obtained ordinary iron-wire screens may be used, but they should be covered with two coats of good paint and frequently repainted. Even so, they will be found more expensive in the end than the best copper-wire screening, which should be used if it can be obtained.

The *size of the mesh* in the wire screening used to protect houses and barracks is a matter of prime importance. It must be close enough to keep out all anopheles mosquitoes, and in regions where yellow fever is endemic or may be introduced the mesh should be small enough to keep out the yellow-fever mosquito, *Stegomyia fasciata*. Nothing is gained by using a screen in which the meshes are closer than necessary, and something is lost, for the closer the mesh the more air is excluded from the room or building, and this is a matter of much importance, especially in the Tropics and subtropics.

A considerable amount of experimental work has been done along this line. As a result of my own observations at Camp Stotsenburg, in the Philippines, where anophelines were very numerous, I concluded that wire netting containing 16 meshes to the linear inch (No. 16, as it is called) excluded all the *Anopheles* tested and that this size netting should be used in the prophylaxis of malaria.³² My observations were confirmed, so far as *Anopheles* are concerned, by Guiteras,³³ Darling,³⁴ and the United States Army Board³⁵ for the Study of Tropical Diseases in the Philippines. This netting was not efficient, however, in excluding the yellow-fever mosquito, as the majority of investigators have shown, and a netting containing 18 meshes to the linear inch should be selected when *Stegomyia* are present, as shown by the experiments of Darling and the Board for the Study of Tropical Diseases.

It is very probable that mosquitoes vary in size, even when full grown, for Goeldi has shown that delayed development during the larval stage, due to various local conditions and a poor supply of food, results in dwarfed adult mosquitoes, and this doubtless accounts

for some of the discrepancies between the results of different investigators as to the efficiency of various sized wire screens.

In 1908 Col. J. R. Kean, Medical Corps, United States Army, sent Dr. Guiteras, in Habana, some 16-mesh wire screening and requested that experiments be made regarding its efficiency in excluding *Stegomyia* mosquitoes. After many very careful experiments Dr. Guiteras reported to Col. Kean that the netting sent kept out *Stegomyia fasciata*, using the following words:

It is my opinion that we may conclude from these experiments that the *Stegomyia calopus* (*fasciata*) can not pass through the wire gauze sent for trial. The gauze is 16-wire mesh—that is, it presents 16 wires or threads to the linear inch.

In 1910 Darling, as the result of his experiments in the Canal Zone, states:

In regions where it is only necessary or desirable to protect against anophelines a No. 16 mesh screening (16 holes to the inch) would answer the purpose, and where, as in this region, it is necessary to protect against some of the smaller varieties, such as *Stegomyia calopus*, a No. 16 mesh would be *practically* safe, but not absolutely so.

He found that none of the anophelines in the Canal Zone could pass the 16-mesh screening, but that some specimens of *Stegomyia fasciata* (*calopus*) could, as well as several species of *Culex*.

Owing to the uncertainty caused by the divergent reports of Guiteras and Darling, the Surgeon General of the Army, in 1912, requested the Army Board for the Study of Tropical Diseases in the Philippine Islands to determine what sized copper-wire gauze would prevent the passage of anophelines and other mosquitoes. The board, as the result of very careful experiments, found that 16-mesh wire gauze was impervious to the following mosquitoes: *Myzomyia rossii*, *Mizorhynchus barbirostris*, *Pyretophorus freerae*, *Mansonia annulifera*, *Mansonia uniformis*, *Culex fatigans*, and *Culex microannulatus*. As *Myzomyia rossii* is as small as any of the anophelines connected with malaria, their observations confirm mine, made in 1905 at Camp Stotsenburg, but they also determined that this same species could be excluded by either No. 12, 14, or 16 mesh screening. In addition they found that *Stegomyia fasciata* (*calopus*) could pass No. 16 mesh netting with considerable ease, and concluded that while the No. 16 mesh will keep out all malaria mosquitoes found in the Philippines its use is inadvisable because it does not keep out the yellow-fever mosquito.

From these various observations it appears to me that in the military service all screening should contain at least 18 meshes to the linear inch, and I believe that it is a mistake to use wire gauze containing more than this number, both because it is unnecessary so far as practical protection goes and because it reduces by just so much the amount of air admitted to a room or building.

Method of screening.—It is a perfectly obvious fact to anyone who inspects screened buildings, either in civil or military life, that much of the screening is so constructed as to defeat, in a measure, the purpose aimed at, i. e., the exclusion of mosquitoes. The use of adjustable or folding screens; the fastening of the screen within the window, necessitating its being raised every time the window is opened; and the use of the single-screened door for quarters and barracks, instead of having double-screened doors with a screened vestibule, are all instances commonly observed of imperfect screening. In barracks that were carefully screened I have observed unscreened ventilators along the ridgepole, which explained fully the presence of numerous mosquitoes within them, an occurrence which had furnished a strong argument against screening in the prophylaxis of malaria to certain individuals who were adverse to this method. Not infrequently, while the first and second stories of quarters are carefully screened, the attic windows will be found unscreened, and mosquitoes thus gain entrance to the quarters. All of these mistakes can be easily avoided and are most important in their effect upon the success of screening against mosquitoes.

The arrangement of screens must vary, of course, with the architecture of the building to be screened, but all window screens should be placed outside window sashes and fitted as closely as possible to the sash, as otherwise mosquitoes may easily get in between the screen and sash, especially when the window is partly open. In fact, to insure perfect protection the entire window should be covered externally by one screen. In this way either the lower or upper sash may be raised or lowered without danger of admitting mosquitoes. Where wire screening is impossible doors and windows may be protected by cheese-cloth screens, which, while not very durable, are satisfactory if carefully watched for the appearance of holes and promptly replaced. All entrances to quarters and barracks where screening is necessary should be protected by a screened vestibule containing two doors instead of a single screened door opening directly into the building, which is the usual arrangement. While this method of screening may be practically sufficient where mosquitoes are not very numerous and malaria is rare, it is necessary to have the double-door entrance in regions where malaria prevails to any extent, especially in barracks, where the doors are opened so frequently. All screened doors should open outward.

Extreme care should be taken that all ventilators be screened, and that screens be frequently inspected and repaired promptly when necessary.

Mosquito nets or bars.—When it is impossible to thoroughly screen barracks and quarters, and for troops in the field, the mosquito net or mosquito bar, as it is often called, furnishes a most valuable means

of protection from the bites of these insects. In the military service the ideal mosquito net would be one that could be used over the bed while the troops are in permanent barracks, and within the shelter tent when in the field. Such a mosquito net has been devised by Capt. Vedder, of the United States Army Medical Corps, and has been adopted by the Quartermaster's Department for issue to the troops. It can be easily folded and carried in the pack when troops are in the field and, if properly made, will undoubtedly be found to be a most valuable aid in preventing mosquito-borne diseases in an army during active operations, besides greatly increasing the comfort of the troops when campaigning in regions where mosquitoes abound.

In barracks where mosquito nets are used there should be a nightly inspection of the nets after taps, to insure their being used by every man and that they are being properly used. Care should be taken to see that each net is tucked under the mattress, instead of hanging loosely around the bed, as otherwise mosquitoes easily gain entrance by crawling or flying between the net and the mattress. During the day the mosquito net should be preferably taken down and folded up on the bed, but may be either tucked in beneath the mattress if the bed is made up or folded over on the portion suspended between the uprights. The greatest care should be taken to keep the net in repair, all holes being darned as soon as they are discovered.

Head nets and gloves.—Head nets and gloves are absolutely necessary in many regions if one is camping or hunting, and the same is true when troops are in the field in such localities. The Quartermaster's Department of the Army issues a very good head net, so fashioned as to fit over the campaign hat, and this will be found of the greatest service, especially to the men on guard, and their use should be insisted upon in malarial localities. I am convinced that if a head net had been worn by men on guard at several of the posts in the Philippines it would have greatly reduced the amount of malaria, for many cases of infection occurred that could be traced to the soldiers being severely bitten during guard duty.

The use of odorous substances on the skin.—Various substances have been recommended for protecting the skin from the bites of mosquitoes. Among the best may be mentioned the oils of citronella, eucalyptus, pennyroyal, anise, camphor, vaseline, and kerosene. As a rule the slight protection afforded by these substances has not led to any extensive use of them in the military service, but there may be circumstances in which the use of one or the other of them may prove of value. Such an instance is quoted by Howard,³⁶ who states that Dr. W. H. Dade, United States Army, found a mixture of one part of bergamot oil to sixteen parts of kerosene very efficacious in preventing mosquito bites when smeared on the skin, and that this

mixture was successfully used by troops when on marches in the Philippines.

For use, as a temporary measure, a mixture of oil of citronella and vaseline, when smeared upon the skin, is very serviceable and is not unpleasant. If liquid vaseline is used, about one part of oil of citronella should be mixed with six parts of liquid vaseline and applied frequently when exposed to the bites of mosquitoes. If liquid vaseline can not be obtained ordinary vaseline may be used, a teaspoonful of the oil of citronella being mixed with two ounces of vaseline.

It is obvious that the use of substances that are only temporary in their protective power and that depend entirely upon the will of the individual for what success they may have, are not of any great value in the prophylaxis of malaria in the military service. Such methods are miserable makeshifts and should be rendered unnecessary by the adoption of more scientific and valuable ones.

The value of screening and the use of the mosquito net has been abundantly proven by practical experiments, beginning with that of Sambon and Low. These investigators, in order to test the truth of the theory that the malarial infections are transmitted by mosquitoes, spent an entire summer at Ostia, a most malarious region in the Roman Campagna, residing in a mosquito-proof hut. During the day the time was spent mostly out of doors, but early in the afternoon the observers retired to their hut and there spent the night. Neither investigator developed malaria, although it was said, by the natives, that to spend one night in that place would result in a malarial paroxysm, and most of the inhabitants who were not protected by screens developed the disease during the summer the observers spent there.

Of the notable instances of the protection from malaria afforded by screening may be mentioned the experiments of Procaccini, in Sardinia, and of Tzuzuki, in Formosa, with military troops; and in the Canal Zone, in the settlements of Gatun and New Gatun, as reported by Orenstein.

Procaccini,³⁷ in Sardinia, reduced the number of cases of malaria among the soldiers from 70 per cent to 57 per cent, in one season, by screening the barracks, and later the percentage was reduced to less than 20 per cent.

In Formosa, Tzuzuki³⁸ tested the efficiency of screening by selecting 115 soldiers of a battalion stationed at Kirun, a most malarious locality, and furnishing them with screened barracks, the remainder of the battalion being unprotected from the bites of the mosquitoes. The 115 soldiers were thus protected from September 21 to December 8, the malarial season, and not a case of the disease developed among them, while, in the same time, among the 750 soldiers not so

protected there developed 319 cases of malaria. The men were confined in the screened barrack from half an hour before sunset to half an hour after sunrise during this time, and wore head-nets and gloves when on service at night.

Orenstein³⁹ gives a most interesting account of the value of screening as illustrated in the settlements of Gatun and New Gatun, in the Canal Zone. Gatun had a population of approximately 4,500, residing in screened quarters, while New Gatun, having a population of about 5,000, had no screened quarters. With this exception the surroundings were identical so far as chances for malarial infection were concerned. Observation extending over three years showed that the malarial incidence for Gatun and New Gatun was as 2 is to 3, the following being the yearly incidence in each settlement:

Year.	Gatun.	New Gatun.
	<i>Per cent.</i>	<i>Per cent.</i>
1909.....	5.35	10.04
1910.....	5.37	9.21
1911.....	8.75	12.59

In his conclusions Orenstein says:

A properly screened dwelling can be depended upon to reduce by at least one-third the malaria incidence in a locality where malaria is endemic.

These instances illustrate how valuable a prophylactic method we possess in screening when it is efficiently applied, but it should be remembered that it is far less valuable than is the abolition of the breeding places of mosquitoes when this can be accomplished. However, as has been repeatedly stated, it is only under exceptional conditions that one is able to entirely rid a locality of mosquitoes, and it therefore follows that screening must always remain one of our most valuable accessory methods in the prophylaxis of these infections. In the field, of course, we must depend entirely upon the use of the mosquito net and quinine in the prophylaxis of malaria among troops, but in permanent posts screening should not be depended upon to the exclusion of other prophylactic measures, but should be used along with them.

Screening of malarial patients.—In concluding this chapter I desire to call attention to the very great importance of screening all malarial patients as long as the plasmodia can be demonstrated in any number in the peripheral blood. It is obvious that if we place the infected individual in a position where mosquitoes can bite him the mosquitoes will become infected, provided *gametes* are present, or the patient may become reinfected by the bite of an infected mosquito. Despite these facts I have several times observed malarial patients in a common ward lying unprotected by a mosquito net,

although the ward was not screened and anophelines were present. It is needless to comment upon such a situation, but it is one that will continue to occur, in isolated instances, until the malarial fevers are considered as infectious diseases and the same precautions taken to prevent their spread as would be taken in the case of yellow fever.

In a carefully screened ward it would be unnecessary, of course, to further screen a malarial patient, but in an unscreened ward this should always be done, and, under such circumstances, it is better to place the patient in a room by himself, the same precautions being taken regarding screening, nor should he be allowed to leave the screened quarters until the plasmodia have either disappeared from his blood or been reduced to a noninfectious minimum. This will, of course, necessitate a microscopic examination of the patient's blood at daily intervals, but it is only by such an examination that we can adequately control the treatment of malaria and render the prophylaxis of the disease effective, so far as the patient is concerned. The screening of every individual in hospital for malarial fever is an absolutely necessary prophylactic measure if one desires to limit the spread of these infections.

CHAPTER V.

PROPHYLACTIC METHODS BASED UPON THE DESTRUCTION OF MALARIA PLASMODIA (QUININE PROPHYLAXIS).

It is obvious that if we can destroy or prevent the development of the malaria plasmodia in infected individuals we will succeed in preventing the disease, as it will thus be impossible for mosquitoes to become infected. This fact is the basis of quinine prophylaxis, which consists in the administration of this drug to all individuals harboring the plasmodia and to all those exposed to the bites of mosquitoes capable of transmitting the plasmodia to man.

Perhaps no method in the prophylaxis of the malarial fevers has given rise to more controversy than the use of quinine as a prophylactic. Enthusiasts in its favor have claimed that the use of this drug alone is all that is needed to eradicate malarial infections from any locality, while its most violent enemies have claimed that it is not only practically useless but even harmful. It is evident that the truth must lie somewhere between these extreme opinions, and it is my belief that quinine prophylaxis is a most valuable method, but one that should not be relied upon to the exclusion of other methods except under conditions that render the combination with other methods impossible.

From the observations of many investigators we know that quinine is capable of destroying the plasmodia while they are in the blood of man, and from clinical observation we know that this destruction is followed by the disappearance of the symptoms of infection and the recovery of the patient. In other words, quinine is a specific in the treatment of malaria, and to deny the efficacy of the same drug, when properly administered, in the prophylaxis of the same disease is absurd. If quinine can destroy the plasmodia after symptoms of infection are present, it will certainly destroy them before they become numerous enough to cause symptoms; but experience has shown that enough of the drug must be given to really destroy the parasites, and that this means that considerable doses have to be taken and have to be continued just as long as the individual is exposed to malaria mosquitoes.

The most ardent advocates of this method of prophylaxis, as Koch, have claimed that by it alone it is possible to rid a community of

malaria, but in practice this result could only be attained where the drug could be administered to every individual in the infected locality for a long period of time, for the prophylactic use of the drug would have to be continued as long as *Anopheles* were present, for just so long would there be danger of the spread of the disease from the mosquitoes becoming infected by imported cases of malaria. Therefore it follows that while quinine prophylaxis is a method of the very greatest value in the prevention of malaria, it can not be depended upon alone, but should be combined with those that have already been described.

The results achieved by this method of prophylaxis have varied greatly in different localities, depending upon the methods of administration and the form of quinine used; the efficiency with which the administration of the drug has been conducted; and the class of people with whom the method has been tried; so that the literature is filled with discordant reports regarding the value of the drug as a prophylactic. Most of the unfavorable reports have been the result of careless application of the method or of lack of control of the people to be benefited. It is obvious that to be successful the drug must be regularly administered, and in the military service this can be done, whereas it might be impossible in civil communities. It is true, however, that even when the method has been given the most careful trial it has failed, in rare instances, to have much effect upon the incidence of malaria; but the experience of numberless observers of the great value of the method when properly applied leaves no doubt of its worth, and the rare failures recorded must have been due to exceptional conditions surrounding the experiments.

Among the arguments that have been urged against quinine prophylaxis may be mentioned the production of haemoglobinuria, harmful physical effect upon the consumer, the difficulty of application, and the danger of producing a resistant strain of the plasmodia by the exhibition of small doses of quinine over long periods of time. As a matter of fact none of these objections are valid, and there is practically no proof that any of them possess actual merit sufficient to forbid the use of quinine in this manner. Haemoglobinuria is sometimes produced by quinine, but very rarely where the drug is taken regularly, and the few instances in which it does occur is no argument against the use of the drug. There is absolutely no scientific proof that the taking of quinine, even in much larger doses than are used in prophylaxis and continued for weeks and months, produces any harmful effect, and even the slight dizziness and roaring in the ears disappear after taking the drug in prophylactic doses for a few days. The danger of producing quinine-fast strains of the

plasmodia in this manner is simply a theory without any proof of scientific value, while the objection regarding the difficulty of applying the method is true of many other prophylactic methods and a poor excuse for its neglect. Certainly in the military service, where men are under absolute control, there can be no excuse for neglecting quinine prophylaxis when it is needed, because it requires careful supervision and therefore entails labor upon the sanitary personnel. Quinine prophylaxis has been found of the greatest service in keeping down the incidence of malaria in some of our permanent posts in the Philippines, and during active operations by troops in the field in malarial regions it will always have to be our chief defense against these infections. It is therefore essential that the medical officer be acquainted with the various methods of applying this form of prophylaxis, which includes a knowledge of the effect of quinine upon the plasmodia; the form and time of administration of the drug; the proper treatment of initial infections, of latent infections, and of *gamete* carriers; and, finally, of the results that may be expected from this method when it is efficiently applied.

Action of quinine upon the malarial plasmodia.—In 1867 Binz demonstrated that quinine in solution killed infusoria with which the solutions were brought in contact, and concluded from this that its effect in malaria was due to the destruction of the cause of the disease. In 1881 Laveran demonstrated that quinine solutions, when added to blood containing the plasmodia, produced immediate cessation of the movements of the plasmodia, while Golgi, Romanowsky, Mannaberg, Zieman, Schaudinn, and others described degenerative changes produced by the drug in the various species of plasmodia. The results of these various observers were often conflicting, and many of their conclusions were founded upon insufficient evidence. In 1910 I⁴⁰ published the results of continued studies upon the effect of quinine on the various species of plasmodia, as shown in both fresh and stained preparations of blood, and these results have since been confirmed by many observers. They may be summarized as follows:

Plasmodium vivax (tertian plasmodium).—In *fresh* preparations of blood obtained after the administration of quinine marked morphological changes are observed in every stage of development, with the exception of the stage just preceding sporulation and in the fully-developed *gametes*. The morphological changes observed in the *living* organism consist in an initial stimulation of amoeboid activity, followed by a decrease in activity, and eventually by cessation of all motion; a granular degeneration of the cytoplasm which has an increased refractive index; fragmentation of the plasmodium, followed by the apparent extrusion of the fragmented organism from the red corpuscle; and an apparent increase in the amount of pigment in those organisms which undergo development in spite of the

quinine. In *stained* preparations the morphological changes are much more apparent, consisting in poor staining reactions of the cytoplasm, while the chromatin of the nucleus stains darker and is stainable long after the cytoplasm has ceased to stain; the prevention of normal increase in the amount of chromatin, as shown by the plasmodia containing much less of this material at certain stages of growth than normal; the prevention of normal division, as shown by plasmodia in which only a portion has divided, thus producing atypical sporulating forms; and sporulating forms in which many of the spores or *merozoites* are devoid of chromatin. Many of the organisms show undoubted evidences of fragmentation in the stained preparations. The changes observed are present at every stage in the development of the organism except when quinine has been given just before sporulation and in the fully-developed *gametes*.

Plasmodium malariae (*quartan plasmodium*).—The changes produced by quinine, as shown in fresh and stained preparations of blood containing *Plasmodium malariae*, are essentially the same as those produced in *Plasmodium vivax*, and while there is some evidence that this species is slightly more resistant than the tertian plasmodium my observations showed that even the older parasites were affected, just as in the case of the tertian species.

The estivo-autumnal plasmodia.—In both species of estivo-autumnal plasmodia quinine produces practically the same changes as in the tertian and quartan species, except that fragmentation is not so frequently observed. The crescentic *gametes* of the estivo-autumnal plasmodia appeared unaffected by quinine so far as any morphological changes are concerned.

My observations proved that the old theory that only the young spores are affected by quinine and that the drug must be in the blood at the time of sporulation is untenable, for while the *merozoites* are undoubtedly more easily affected by the drug than the older forms, the changes in morphology of the *schizonts* almost up to the time of sporulation demonstrate conclusively that quinine causes degeneration in even the older plasmodia. Recent observers have confirmed my observations regarding the action of quinine upon the later stages in the development of the plasmodia and upon the morphological changes that are produced in these organisms by the drug. Rieux⁴¹ studied the action of the drug upon the tertian plasmodium and found that not only were the young *schizonts* affected, but that if the drug was commenced 24 hours after the paroxysm when the *schizonts* were half grown many of them were arrested in development and fragmentation occurred in a considerable percentage. He found that the sporulating bodies were not affected, but that the continued use of quinine caused degeneration of the *gametes* and eventually a disappearance of these forms from the peripheral blood. Bil-

let's⁴² observations confirmed those of Rieux; and James⁴³, in the Canal Zone, obtained the same results from his studies of the action of quinine upon the plasmodia.

From these observations it is evident that quinine is capable of affecting the plasmodia in practically every stage of their development, and it follows that the best results will be obtained in prophylaxis and treatment if the drug is present in the blood continually. Thus, I am a firm believer in divided doses at regular intervals in the treatment of all forms of malarial infections, except, of course, the pernicious cases, where a large dose of the drug must be administered at once; and in the prophylaxis of the disease I believe that better results will be obtained if quinine be given in morning and evening doses rather than in one dose during the 24 hours. The object desired is to have a maximum quantity of the drug continually present in the blood, and the nearer we approach this ideal the better will be our results with the quinine prophylaxis of malaria. In the field, of course, it would probably be necessary to depend upon one dose, given either in the morning or evening, but in camps and posts a morning and evening administration of quinine could be arranged and enforced in the vast majority of instances.

The form of quinine to be used in prophylaxis.—Until quite recently we have been very ignorant regarding the exact fate of quinine after ingestion and the salts or compounds of the drug best fitted for use in the prophylaxis and treatment of malaria. The researches of Gaglio, MacGilchrist, Webb, and others have demonstrated that many of our ideas regarding the pharmacology of this drug were erroneous and have resulted in new points of view as to its use in malarial infections. Thus, the old idea that the salts most soluble in water should be preferred, because they would be more readily absorbed has been proven incorrect, for Gaglio has shown that the insoluble salts and compounds are as well absorbed as the most soluble salts of the alkaloids, and that the drug is absorbed mostly in the intestine combined with the biliary acids and carbonic-acid gas. It has also been shown that if the drug is taken daily it accumulates in the blood up to about double the initial dose, and that it is eliminated through the kidneys, elimination never taking longer than three days, no matter how administered or what preparation is administered. The least soluble preparations have been found the best tolerated by the stomach and intestines, because of their more gradual absorption, and MacGilchrist has shown that the *acid* salts are strong hemolytic agents outside the body and are, therefore, less safe than the neutral salts or quinine alkaloid itself.

In the selection of the form of quinine to be used in prophylaxis all of the points enumerated are of importance, owing to the length of time that the drug must be administered. If there is serious dan-

ger of the production of hemoglobinuria by the constant administration of the acid salts of quinine they should not be used; but, as a matter of fact, there is no evidence that the prophylactic use of any of the acid salts has resulted in the production of any appreciable amount of hemoglobinuria, so that it may be stated that they may be used in prophylaxis without danger. It should also be remembered that the proper treatment of initial malarial infections and of "carriers" of the disease are most important prophylactic methods, so that the consideration of the form of quinine to be used in prophylaxis must not only take into consideration what form can be used most successfully in preventing the disease, but also what form is most efficient in curing those already infected.

Of the many salts and compounds of quinine that have been used in the prophylaxis and treatment of malaria I believe that, from a military standpoint, the only ones deserving of consideration are the sulphate, the dihydrochloride, tannate of quinine, and the quinine alkaloid. Of these the sulphate and the dihydrochloride (hydrochlorsulphas) are on the supply table, and, in my opinion, are all that are needed in the prophylaxis and treatment of malaria in the Army, although for prophylactic use the tannate might be adopted with benefit.

This is the salt that has been so successfully used in Italy in the prophylaxis of the disease and which Celli regards as the ideal one for this purpose, owing to the fact that it is almost tasteless, is well tolerated by the stomach, is absorbed more slowly, and is more completely oxidized than other salts of the drug. For the treatment of those already infected it is inferior to the sulphate or dihydrochloride. MacGilchrist⁴⁴ has recently urged the adoption, both for prophylaxis and treatment, of the amorphous precipitated quinine base or alkaloid for the following reasons: 1. It is sparingly soluble in water and almost tasteless. 2. Absorption is as quick and complete as after the administration of quinine salts. 3. Being pure quinine it represents quinine in the smallest bulk and weight. 4. Being non-hemolytic it is safer. 5. Low cost. If further experience shows that these contentions are correct it would appear as though quinine alkaloid possesses advantages over salts of the drug, but experience has shown that the sulphate of quinine can be relied upon in prophylaxis and treatment in the military service, and there would seem to be no great advantage to be gained by carrying on the supply table several preparations of the drug. So far as the danger of the sulphate producing hemoglobinuria is concerned, the statistics of the Army demonstrate that it is infinitesimal, for despite the immense amount of this salt that has been used in the prophylaxis and treatment of malaria among our soldiers since 1898, hemoglobinuria has been so rare as to be a medical curiosity.

Methods of administration and dosage.—In discussing this question two problems must be considered, i. e., the best method of administration of quinine to prevent infection and the dose required and the best method of curing initial infections and carriers of the disease. The latter will be discussed under a separate heading, and I will here give the various methods of quinine prophylaxis that are intended to directly protect the individual from infection by the plasmodia.

All methods of quinine prophylaxis may be divided into two classes—i. e., those in which the drug is administered daily and those in which the drug is administered at larger intervals. The latter methods are all aimed at doing away with the irksome daily administration, but all have the fault that one must remember the days upon which quinine should be given, with the result that many doses are missed, while there is good evidence showing that none of them are as effective in prophylaxis as the daily administration of the drug.

The following are the chief methods of using quinine as a prophylactic, together with the doses advocated:

Celli's method.—The daily administration to adults of 40 centigrams (6 grains) of quinine, 20 centigrams in the morning and 20 centigrams in the evening. To children, 20 centigrams (3 grains), 10 centigrams in the morning and 10 centigrams in the evening.

Sergent's method.—The administration in one dose, daily, of 20 centigrams (3 grains) of quinine.

Zieman and Nocht's method.—The administration of 1 gram (15 grains) of quinine every fourth day, given in 0.25-gram (4-grain) doses.

Plehn's method.—The administration of 1 gram of quinine (15 grains) every seventh day.

Koch's method.—The administration of 1 gram (gr. xv) every sixth and seventh, seventh and eighth, eighth and ninth, or ninth and tenth days, according to the severity of the infections present.

While our choice of the exact method of giving quinine as a prophylactic will be influenced in the military service by local conditions, I believe that the method of Celli, supported as it is by years of success in practice, will be found the most efficient one for adoption by troops campaigning in a malarial country. The giving of the drug in morning and evening doses would probably often have to be replaced by the practice of giving the entire amount in one evening dose, but the *daily* administration of the drug I believe to be essential to the success of malarial prophylaxis in an army in the field. For such service, then, I would recommend the administration of 40 centigrams (6 grains) of quinine sulphate to *every soldier every day*, preferably in an equal morning and evening dose, but if this is impracticable, in a single dose at evening.

In regard to malaria prophylaxis in semipermanent camps and permanent posts, the daily administration of the drug in the dose indicated, with the proper reductions in dose in women and children, will likewise be found the most efficient method; but if for any reason this method can not be adopted, the administration of 1 gram (15 grains) on the evening of every third day should be preferred to any other of the *interrupted* methods of quinine prophylaxis. The reason for this is evident. It has been shown that all of the quinine taken at any one time is entirely eliminated within 72 hours, so that if an interrupted method of giving the drug is adopted it should be one in which there is no time when the blood is entirely free, and this can only be accomplished by selecting one in which the dose is repeated within 72 hours. While with the method advocated there will be a period of an hour or so in which the blood is free from quinine, this can be disregarded in practice. This method has the advantage also that it is easier to remember to take the dose of quinine every other day than at the fourth, ninth, and other days required by some of the methods of quinine prophylaxis already mentioned. The more simple we make our methods of prophylaxis the better they will succeed, especially with soldiers, and for this reason I do not favor the complicated methods advocated by some authorities in which quinine is to be taken at irregular intervals. The two methods outlined here will be found efficient in practice, the choice, if possible, resting with the daily administration of the drug.

The administration of smaller doses of quinine than those advocated in the prophylaxis of malaria should be discontinued both because smaller doses are inefficient and because if there is any danger of the production of quinine-fast strains of the plasmodia they are much more apt to be produced by doses of 1 and 2 grains of quinine daily than by the amount recommended. Personally, I see no proof that such strains have ever been developed, and while the theory is fascinating, especially in the explanation of resistant infections and relapses, there is no scientific evidence that such forms of the plasmodia actually exist. Therefore, my objection to smaller doses of quinine than those mentioned is based almost entirely upon my conviction that such doses are insufficient to prevent infection and are practically useless in the prophylaxis of malaria.

It should be distinctly understood that the dosage of quinine recommended to prevent malarial infections in the healthy has nothing whatever in common with that employed in preventing relapses in the infected or in the treatment of "carriers" of the infection. These subjects will be shortly referred to, but the experience of Celli, in Italy, absolutely demonstrates that quinine

given in the manner here recommended does prevent malarial infections, although some authors, as Thomson, would have us believe that much larger doses are necessary. It is true that larger doses have to be used in the treatment of the infected, but for the prevention of the disease in the uninfected the doses recommended will be found efficient in the very great majority of instances.

As a prophylactic quinine must be administered by the mouth. When given in this way it may be administered in the form of a solution, pills, tablets, capsules, wafers, troches, and confections. In the military service the solution and tablets are about the only practicable forms where large bodies of men are to be treated, and in the field the cheapest and most useful method of dispensing the drug is in the form of tablets containing the required dose. Nowadays excellent tablets may be obtained, but even so, the utmost care must be used to see that quinine is really being supplied and that the tablets are soluble. We are all familiar with the insoluble quinine tablet and pill, but at the present time the tablets upon the market are satisfactory in this respect and will be found efficient.

The solution of quinine may be employed in camps and posts and possesses the advantage that the drug is more readily and quickly absorbed and there is little chance of the soldier evading the taking of the dose. The solution is prepared by dissolving 0.32 grams (5 grains) of quinine sulphate in 4 c. c. (1 dram) of distilled water to which one drop of concentrated hydrochloric acid has been added. A large amount of this solution can be made up at one time, as it keeps well, and can then be dispensed as needed.

In order to be effective the administration of quinine as a prophylactic must be under the direct personal supervision of a medical officer, and this duty should not be delegated to any subordinate, but should be considered one of the most important *personal* duties that the sanitary officer is called upon to perform. Many of the reported failures of quinine prophylaxis in troops have been due to the relegation of this duty to careless subordinates and their inefficient application of the particular method selected, with the result that the method has been condemned without a fair trial.

The proper treatment of malarial infections and of "carriers" in the prophylaxis of the disease.—It is well known that after an attack of malarial fever has persisted for from 8 to 10 days or more, provided insufficient quinine has been given, there develop in the blood of the infected individual forms of the plasmodia known as *gametes*, which are capable of infecting anopheline mosquitoes, and eventually of rendering these insects capable of transmitting malaria to man. It is evident that if these forms could be prevented from developing mosquitoes could not become infected and malaria could not be transmitted. It follows, therefore, that if quinine will prevent the for-

mation of *gametes*, and it will, every patient presenting these forms in the blood is an evidence of the improper treatment of his infection by his physician, provided, of course, that they were not present when he was first seen, as the result of a latent or very mild infection. As a matter of fact the vast importance of the proper treatment of the infected in the prophylaxis of the malarial fevers has never been fully realized by the profession or even by officers engaged in sanitation in malarial regions, for there is nothing more common, in many such localities, than to see *gamete* carriers going about their daily occupations without having received any advice regarding treatment and wholly ignorant that they are a danger to the people of the community in which they reside. In much of our prophylactic work in the military service this phase of the subject appears to have been entirely overlooked and it is because of their importance in the prophylaxis of malaria that the subjects that follow are here discussed.

In any thorough campaign against the malaria fevers the discovery and treatment of latent infections, the treatment of *gamete* carriers, or carriers of malaria, and the proper treatment of initial and recurrent cases of the disease are fully as important as other methods of prophylaxis, and yet, until recently, these subjects have been looked upon as being practically outside the province of the sanitarian and of preventive medicine. As a matter of fact, the prevention of "carriers" of any infection and the treatment of those who have become carriers is one of the most important function of preventive medicine, while the discovery of latent infections and their treatment would logically appear to be the first step in the prevention of any disease. This has been found to be true in typhoid fever, diphtheria, entamoebic dysentery, cholera, and other infectious diseases and it is equally true in the malarial infections.

The discovery and treatment of latent infections.—By a latent malarial infection we understand one in which the plasmodia may be demonstrated in the peripheral blood, but in which no clinical symptoms of sufficient gravity to attract attention are observed. The term includes both those instances in which no definite symptoms of malaria have ever been observed, and those cases in which the disease is latent between recurrences.

It is well known that in malarial localities a considerable proportion of individuals in apparently fair health are "carriers" of the plasmodia, and that the natives of these regions possess an acquired immunity to the usual effects of the malaria toxin, although the plasmodia may develop in such individuals and be demonstrable in the blood. It has also been shown that insufficient treatment of

malarial infections results in the production of carriers of the disease and that many patients discharged as cured from the hospital will be found to have plasmodia in their blood for long periods before a relapse occurs, and that many of these become efficient "carriers" of the disease owing to the development of *gametes* during these latent periods.

Proportion of latent infections.—The proportion of latent infections varies greatly in different localities as is shown by the tables that follow, but it may be stated that such infections occur in a considerable percentage of both children and adults in every region in which malaria is endemic to any extent, and that the discovery and treatment of these infections is of great importance in the prophylaxis of malaria in such regions. In the following tables the percentage of malaria in the cases recorded was determined by the finding of the plasmodia in the blood, which is a much more accurate method than the splenic index which will be discussed later.

Koch,⁴⁵ to whom we owe practically the first observations along this line, concluded, from his work in Africa, that latent malarial infections were almost entirely confined to children, but further observations have shown that latent infection is very frequent in adults in malarial localities, and that the true index of the prevalence of the disease in any region can only be determined by the examination of the blood of both children and adults. The following tables give the results of the principal investigations in regard to the prevalence of latent infections in the natives of malarial regions:

TABLE I.—*Thomas's observations in Manos, North Brazil.*⁴¹

Age.	Number examined.	Number infected.	Percent-age.
6 months to 2 years.....	9	3	33.3
2 years to 5 years.....	38	20	52.6
5 years to 8 years.....	51	28	54.9
8 years to 10 years.....	59	27	45.7
Total.....	157	78	49.6

James,⁴⁷ in India, found the percentage of latent infection in native children to vary greatly in different localities. At Mian Mir children up to 2 years of age showed 80 per cent infected; up to 5 years, 66 per cent; up to 10 years, 50 per cent, while children older than 10 years showed no infection. At Ennur children up to 3 years years of age showed 65 per cent infected; up to 5 years, 51 per cent; up to 10 years, 46 per cent, and up to 15 years, 16 per cent.

At Camp Stotsenburg, in the Philippine Islands, I⁴⁸ have examined the blood of 45 native adults and 180 native children, all of whom at the time of examination were free from symptoms of mala-

ria. The following table gives the results of these examinations, but only includes 147 of the children, as only in this number could I obtain the exact age.

TABLE II.—*Craig's observations at Camp Stotsenburg, Island of Luzon, Philippines.*

Age.	Number examined.	Number infected.	Percentage.
1 to 5 years.....	40	30	75
5 to 10 years.....	54	20	37
10 to 15 years.....	53	13	24.5
Adults.....	45	28	62.2
Total.....	192	91	47.3

The large percentage of adults showing latent infection in this locality was contrary to the reports of Koch, James, and others that only the children in malarial localities suffered from latent infection, but my observations have since been confirmed by numerous observers, as is shown in the following tables:

TABLE III.—*Ollwig's observations in Dutch East Africa.*⁴⁹

Age.	Number examined.	Number infected.	Percentage.
Under 1 year.....	93	33	35.4
1 year to 5 years.....	220	83	37.7
5 years to 15 years.....	971	109	11.2
Adults.....	650	105	16.1
Total.....	1,934	330	17.0

TABLE IV.—*Sorel's observations on the Ivory Coast, Africa.*⁵⁰

Age.	Number examined.	Number infected.	Percentage.
Under 1 year of age.....	134	75	56
1 year to 5 years.....	253	141	56
5 years to 15 years.....	328	125	38
Over 15 years (adults).....	275	110	43
Total.....	990	451	44.4

TABLE V.—*A. Plehn's observations in Kamerun, West Africa.*⁵¹

Age.	Number examined.	Number infected.	Percentage.
Under 2 years.....	18	17	94
Between 2 and 5 years.....	26	24	92
Between 5 and 10 years.....	40	34	85
Adults.....	43	26	60
Total.....	127	101	79.5

TABLE VI.—*Sergeant's observations in Algeria, Africa.*⁵²

Age.	Number examined.	Number infected.	Percentage.
1 to 5 years.....	1,316	267	20.2
6 to 10 years.....	1,360	326	23.9
11 to 15 years.....	933	272	29.1
Adults.....	2,471	722	33.3
Total.....	6,080	1,587	26.1

In Pabna Hope found that while 922 adults showed latent infection only 862 children showed the plasmodia, thus demonstrating that in this locality the adult latent malaria ratio was higher than the ratio in children.

If we consolidate the data relating to latent malarial infection obtained from different localities it will be found that the percentage of infection varies but little in adults or children in badly infected localities, as is shown by the following table compiled from the observations of the authors quoted and from my own:

TABLE VII.—*Consolidated table showing the prevalence of latent malarial infections at various ages.*

Age.	Number examined.	Number infected.	Percentage.
1 to 5 years.....	1,684	502	29.8
5 to 10 years.....	1,645	463	28.1
10 to 15 years.....	1,390	437	31.4
Adults.....	4,931	1,139	23.0
Total.....	9,650	2,541	26.3

From these observations, which comprise only a few of the many relating to latent infections, one may gather an idea of the immense importance of this subject in the prophylaxis of the malarial fevers, where anopheline mosquitoes can not be reduced beyond a noninfectious minimum, and as this is true of the majority of places in the Tropics the recognition and treatment of the latent infections among the surrounding native population is absolutely necessary in order to control the disease. This was demonstrated at Camp Stotsenburg, where a very large part of the infection present among the troops was contracted from the latent infections present in the native population surrounding the post. Although the greatest precautions were taken regarding the abolition of the breeding places of mosquitoes and the use of mosquito nets in the barracks, the troops suffered greatly from malaria, and it was not until the examination of the blood of the natives in the surrounding barrios showed that practically 50 per cent of them were "carriers" of the infection that the prevalence of malaria among the troops was fully explained. It is undoubtedly true that a quarantine against the little barrios in close

proximity to this post would have very greatly reduced the amount of malaria among the troops at that time, as the observations upon the latent infections in the natives proved conclusively that much of the malaria existing among the troops at Camp Stotsenburg was due to infection received while visiting the barrios and that any efforts to limit the disease in the post must take this condition into account. It will thus be seen that latent infection among the natives in a country occupied by troops is a most important factor in the spread of malaria, and that the discovery and, if possible, the treatment of these infections, is necessary in the prophylaxis of the disease.

Not only are the natives in a malarial region a source of danger to troops, but latent infections among the soldiers themselves furnish a constant menace to the health of the command. Improperly treated cases of the disease almost always become "carriers" of the infection, and it is just as important to recognize latent infection among the troops as it is among the native population. As an illustration of this I may mention the results of an examination of the blood of a company of United States soldiers returning from the Philippine Islands in 1902.

In August, 1902, Company H, Sixteenth Infantry, United States Army, reached San Francisco from the Philippine Islands, having served in the Cagayan Valley, a notoriously malarial region in those islands. On August 16, 1902, this company, out of a total strength of some 60 men, had 14 men in hospital suffering from malarial infection, all having had chills since arrival in the United States. On account of this large proportion of sick in hospital, I believed it would be advisable to make a blood examination of the entire company, and accordingly, on August 17 and 18, I examined the blood of every man on duty in Company H, with the following results: Of the 47 men who were doing duty and were apparently in good health, I found that 27, or over 57 per cent, showed some form of malaria plasmodium in their blood. Of these 27 men, 25 were infected with the estivo-autumnal plasmodia and 2 with the tertian plasmodium. Of these men no less than 13 showed crescents or *gametes*, and were, therefore, carriers of malarial infection, while the remainder showed the forms observed in the human life cycle of the plasmodia. It is unnecessary to discuss the importance, from a prophylactic standpoint, of such a body of men, should they have entered a territory in which anopheline mosquitoes were present but in which malarial infections were absent, nor is it necessary to speak of the importance of properly treating such infections in the prevention of the disease. One thing, however, should be emphasized, and that is that had these men been properly treated in the beginning their infections would have been cured and they would not have become carriers of the disease.

Methods of recognizing latent infections.—We have two methods of recognizing latent malarial infections—the examination of the blood and the palpation of the spleen, which is generally enlarged. Of these methods the examination of the blood is altogether the most accurate, although it involves considerable labor, and for this reason is often neglected. The splenic index, as it is called, is often a valuable guide as to the prevalence of malaria in a locality, but this organ is enlarged from other causes than malaria, and this fact often renders the splenic index misleading. In addition, the enlargement of the spleen may simply mean past infection and does not always indicate that treatment is needed at the time of examination. Where blood examinations can not be made, the splenic index must be relied upon; but in the military service, when it is desired to ascertain the actual amount of malaria present, blood examinations of the natives should always be depended upon, and in the prophylaxis of the disease among the troops a blood examination should always be made before patients are returned to duty after an attack of malaria and in any effort to discover latent infections among the men. Every medical officer should be familiar with the appearance of the various species of malaria plasmodia in both fresh and stained preparations of blood, and for this reason the detailed description of these organisms and the methods of demonstrating them have been given in the opening chapter of this contribution. It is true that much time and patience is required in making an estimate of latent infection in a native population and in discovering these infections in the men of a command, but the importance of the procedure is so great that it is time well expended, and the labor involved is no excuse for the neglect of this very valuable prophylactic measure.

The treatment of latent infections.—Immediately upon the discovery of latent infections treatment should be commenced, and the treatment will vary with the particular species of plasmodium present, and whether *gametes* have developed. The treatment of the latter class of cases will be considered in the following section dealing with “carriers” of malaria, as it differs widely from that necessary in those individuals in whom only the forms of plasmodia concerned in the human cycle of development are present, comprising approximately 50 per cent of all latent infections.

The proper treatment of the class of cases just referred to is imperative if one wishes to prevent the development of *gametes*, for in the vast majority of such cases it is only a question of time before they will become “carriers” of the disease, for while *gamete* formation may be delayed it undoubtedly occurs at some time in the vast majority of latent infections, as has been repeatedly shown by blood examinations in individual cases. Fortunately, these infections, before the formation of *gametes*, are even more amenable to treatment

than acute infections, and I believe that I am safe in stating that every one of them can be cured if properly treated.

So far as prophylaxis is concerned, the object of treatment in latent infections in which only the forms of plasmodia belonging to the human life cycle occur, is to prevent the individuals harboring these forms from *becoming* carriers of malaria, as they will be if *gametes* are allowed to develop. As these individuals are not infective to mosquitoes, because *gametes* have not yet developed, there is no necessity for isolating and screening them, and as they present no symptoms the treatment may be pursued while they are attending to their ordinary avocations. In the case of soldiers it is not necessary to relieve them from duty, provided arrangements can be made for them to receive treatment at stated intervals under the direct supervision of a medical officer, so that the treatment of these infections need not interfere materially with military duties nor seriously deplete the strength of the command. It goes without saying that the discovery and treatment of latent malarial infection, so far as the military service is concerned, must be confined to semipermanent camps and permanent posts, as these measures would be impracticable in active service in the field where protection from malaria must depend upon quinine prophylaxis, the use of the mosquito net, and other measures for protecting the men from the bites of these insects.

While in civil life the treatment of latent infections due to the tertian and quartan plasmodia differs from that of infections due to the estivo-autumnal plasmodia, in that smaller doses of quinine are used for the former infections, it will be better in the military service to adopt a scheme of treatment that will cure all cases and that can be easily remembered by the individuals concerned. The following treatment of latent infections *in which gametes are not present in the blood*, while it may err upon the side of slightly too much quinine in the case of tertian and quartan malaria will be found efficient in infections due to the estivo-autumnal plasmodia. It should therefore be adopted if a single rule of treatment of all latent cases is to be followed, and this would appear to be the ideal method in the case of troops. This method of treatment is as follows:

Every individual showing plasmodia in the blood (unless *gametes* are present) should receive 2 grams (gr. 30) of quinine daily until the plasmodia have disappeared from the peripheral blood. In most instances this will occur within three or at most four days. After the plasmodia have disappeared quinine should be given in daily doses of 1 gram (grs. xv) for two weeks, and for two weeks thereafter a daily dose of 0.65 gram (grs. x) should be administered. After this the usual prophylactic dose of 0.40 gram (grs. vi) should be given daily for at least two months.

The method of administration as regards time will often depend upon military necessities, but it is best to give the quinine on retiring, or if it can be arranged the larger doses may be divided into equal morning and evening portions. The method may be easily remembered if stated as follows: Thirty grains of quinine daily until the plasmodia disappear, 15 grains daily for two weeks, 10 grains daily for two weeks, and 6 grains daily for two months.

If this method of treating these infections be faithfully carried out, it will be found that latent malaria will disappear and that none of the cases will become *gamete* carriers unless these forms of the plasmodia develop within a day or two of commencing treatment, when the treatment to be advised for destroying these forms should be adopted.

The time to treat latent malarial infection is before the development of *gametes* because these forms are very much more resistant to quinine than are the forms concerned in the human life cycle and because the isolation and screening of the infected individual is necessary while treatment is being pursued if *gametes* are present. Any considerable number of *gamete* carriers in a military post is a reflection upon the treatment of malaria adopted by the responsible medical officer, and is proof positive of the improper treatment of acute and latent malarial infections.

The treatment of "carriers" in the prophylaxis of malaria.—One of the most important, and yet one of the most neglected, prophylactic measures against the spread of malarial infection is the discovery and treatment of "carriers" of the disease. It has already been stated that after a malarial infection has persisted for from 8 to 10 or more days in man, forms of the plasmodia develop that are capable of further development in the mosquito, and that these eventually render this insect infective to man. These forms are called "*gametes*" and they can easily be detected in the blood by microscopic examination. It is obvious that if these *gamete* carriers can be discovered and the *gametes* killed, these individuals will cease to be infectious to the mosquito; but despite this knowledge, this most important method of preventing malaria has been all but neglected, even in the military service. It has been shown that the *gamete* carriers can be rendered harmless by proper treatment with quinine and that it is feasible to employ the drug in this way as a prophylactic against the disease.

Discovery of gamete carriers.—The treatment of *gamete* carriers, or carriers of the malarial infections, depends upon their discovery, and this depends entirely upon the examination of the blood of individuals residing in malarial localities. The control of the treatment of acute and recurrent infections by frequent examination of

the blood will prevent their being discharged from hospital before the *gametes*, if present, are reduced to a noninfectious minimum, but there are many individuals in malarial regions who are *gamete* carriers and who have never suffered from marked symptoms of malarial disease. Therefore it is necessary not only to control the treatment of acute infections by blood examinations, but to examine the blood of the apparently healthy if we wish to discover all *gamete* carriers and render them harmless.

The time of occurrence of gametes.—It is universally admitted that *gametes* do not develop in patients who have been properly treated with quinine, and that in untreated cases they do not develop until the infection has persisted for several days. The length of time required for the development of the *gametes* of the estivo-autumnal plasmodia varies from 8 to 15 days, the usual period being about 12 days. In other words, an estivo-autumnal infection must have persisted for nearly two weeks before we can expect to find the crescents, or *gametes*, in the peripheral blood. The *gametes* of the tertian and quartan plasmodia appear in the peripheral blood of man in from 7 to 10 days after the onset of definite symptoms of infection.

Although the time given is based upon the appearance of the *gametes* after symptoms are noted, it should be remembered that these forms are often found in individuals who have never shown marked symptoms of infection, and we may find these forms present on what appears to be the very first day of an obvious infection.

However, in the vast majority of patients, *gametes* do not appear in the blood until several days after the appearance of definite symptoms of malaria, and this fact is of great importance in the prophylaxis of the disease, for proper treatment will prevent their development and thus prevent the infection of the mosquito. The fact is also of significance in explaining the origin of these forms, and would appear to indicate that *gametes* are not introduced into man by the mosquito, but that, as Schaudinn believed, they are differentiated during the human life cycle of the plasmodia as the result of the reaction of the human organism to the parasites, and certainly all the evidence we possess points to this conclusion.

The percentage of individuals showing gametes.—As only those individuals in whom the sexual forms, or *gametes*, occur are capable of infecting the mosquito, and thus, indirectly, of infecting man, it is of interest to know how large a percentage of our malarial patients become "carriers." It may be stated at once, that unless properly treated, practically 80 per cent will probably become capable of infecting the mosquito, but the statistics here given are based upon actual observations.

The number of malarial patients becoming "carriers" will vary, of course, in different localities, owing to conditions favoring the persistence of the infection, the type of infection present, the thoroughness with which treatment is carried out, and perhaps other conditions with which we are yet unfamiliar. This subject has been most carefully studied in the estivo-autumnal infections, and the data we possess show beyond question that the percentage varies greatly and that the incidence of *gametes* in the peripheral blood can not be taken as a true index of their actual occurrence in the body, for in many instances it has been demonstrated that blood obtained by splenic puncture was rich in *gametes* when none could be demonstrated in the peripheral blood. In my own experience *gametes* have been observed in a little over 33 per cent of all the cases of estivo-autumnal malaria whose blood I have examined, but I am convinced that a longer search in the majority of these patients would have considerably increased this percentage. In the instances of recurrent infections in Filipinos, which I have had the opportunity of studying, crescents occurred in fully 80 per cent and were as numerous in the adult Filipino as in the children. Most of these cases had received little or no treatment, and for this reason, I believe, the percentage is practically what would be observed in untreated individuals. From my own observations, I believe that at least this percentage of cases of estivo-autumnal malaria will become "carriers" unless they are properly treated.

We possess but little data regarding the percentage of patients infected with the tertian and quartan plasmodia in whom *gametes* develop, but, in my own experience, I have found that these forms occur in about 50 per cent of the cases of infection admitted to hospital and in about 30 per cent of latent infections.

For practical purposes, an individual who does not show *gametes* in the peripheral blood must be considered as incapable of transmitting infection to the mosquito, but this conclusion should not be arrived at from a cursory examination of the blood but only after the most careful examination of at least two specimens, taken at different times, and, if possible, on different days, for these forms are often numerous in the peripheral blood at one time and practically absent at another.

Estimation of the number of gametes in prophylaxis.—The observations of Darling and Thomson have shown that a certain number of gametes must be present in the peripheral blood in order to render the infected individual infective to the mosquito. In a very ingenious series of experiments Darling⁵³ arrived at the conclusion that the peripheral blood must contain at least 12 crescents per cubic centimeter, or more than 1 per 500 leucocytes, in order to be infective to the mosquito, and that patients whose blood contains this number

should be regarded as gamete-carriers and kept in hospital until, by the administration of quinine, the number is reduced below this minimum. The number of *gametes* present is easily ascertained by comparing the number with the number of leucocytes counted in a stained smear, properly prepared, and while there may be numerous theoretical objections to this method of estimating the number actually present, I believe that the rule is a safe one to follow in prophylactic work, and that if it were faithfully followed it would result in a great reduction in the amount of malaria in any locality.

The effect of quinine upon the gametes.—No morphological changes have been noted by myself in fully developed *gametes* of any of the species of malarial plasmodia after the administration of quinine, and the observations of Marchiafava, Gualdi, Martirano, Darling, and others, have shown that these forms are capable of development in the mosquito, even though this drug has been administered for weeks in large doses. Therefore the consensus of opinion at the present time is that after the *gametes* of the various species of plasmodia are fully developed quinine has no action upon them, so far as rendering them incapable of developing in the mosquito. This is not true, however, in the early intracorpuseular stages of the development of the *gametes*, for I have observed marked changes in their staining reactions after the administration of quinine which lead me to believe that this drug is capable of injuring them and probably of entirely preventing their development.

The observations of Darling⁵⁴ and Thomson⁵⁵ have shown that the administration of large doses of quinine, continued over a period of about three weeks, will result in the reduction of the number of *gametes* in the peripheral blood to a noninfectious minimum, i. e., to less than 1 to 500 leucocytes, and, according to Thomson, to less than 1 per cubic centimeter. This reduction is considered by Thomson to be due to the destruction of the asexual parasites from which the crescents arise, but I am of the opinion that it is due to the destruction of the *gametes* themselves, during their early intracorpuseular stages of development, for reasons already noted.

The treatment of gamete carriers in prophylaxis.—The importance, from a prophylactic standpoint, of rendering *gamete* carriers harmless, is self-evident, and the researches of the observers mentioned have shown us how this may be accomplished. No patient suffering from malaria should be returned to duty until a microscopical examination of the blood shows that *gametes* are absent, or at least are present in numbers smaller than 1 to every 500 leucocytes. If these forms are present in a larger number than this, it is necessary to continue quinine in large doses, and both Darling and Thomson, as the result of their very careful observations, state that the drug should be given in doses of 2 grams (grs. xxx) per day for about

three weeks before one can expect to reduce the number of *gametes* to a safe margin in the majority of cases they have observed. Of course the dosage may be reduced if the *gametes* are present in small numbers, and Thomson recommends a daily dose of 1.3 grams (grs. xx) for the same period of time in such cases.

The same treatment should be given the *gamete* carriers discovered in making a malarial survey and in whom the infection is latent. In the case of troops, every soldier, who is not in hospital and who shows *gametes* in his blood, should be placed there at once, carefully screened, and treated with quinine in the dosage recommended until his blood shows less than 1 *gamete* per 500 leucocytes. There is no other alternative than the thorough treatment of these "carriers" in camps and posts where anophelines can not be eliminated, and the only way to treat them is to confine them in a screened room and to give quinine in large doses until the blood examination shows that it is safe for them to return to duty. If allowed at liberty, these men furnish a constant source of infection to the remainder of the garrison and render other prophylactic measures less useful than they otherwise would be.

The treatment of *gamete* carriers must be controlled by the frequent microscopic examination of the blood, as in no other way can we be sure when the patient is ready to be returned to duty. Here, again, we see the absolute necessity of every sanitary officer being familiar with the use of the microscope in the diagnosis of malaria and of the morphology of the plasmodia and the methods of demonstrating them.

The treatment of initial and recurrent infections in prophylaxis.—In the preceding discussion of *gamete* carriers it has been shown that the *gametes* do not develop until the infection has lasted for several days; that after they do develop, the patient becomes a "carrier" of malaria; and that in order to render him harmless he must be put upon sick report, confined in a screened room, and given large doses of quinine for practically three weeks. This is absolutely necessary from a prophylactic standpoint, but a still more important prophylactic measure is to so treat malarial infections that the *gametes* do not develop. Every *gamete* carrier is an evidence of either improper treatment or of no treatment, and if of improper treatment, an evidence of ignorance or carelessness on the part of the attending physician. In other words, the proper treatment of the initial attack of malaria would have prevented the formation of *gametes*, and there would have been no "carrier" of the disease. In the prophylaxis of malaria the proper treatment of acute infections is of vast importance, an importance that has not been realized by the profession, and it is sad, but true, that a very large proportion of the malaria present in the majority of localities is directly due

to the improper treatment of infections coming under the direct care of the practitioner. The practice of regarding a malarial infection as cured because the symptoms have disappeared is responsible for most recurrences and for the development of "malaria carriers," and it is too often the case that a patient is allowed to resume his work without a blood examination having been made or any directions given regarding the continued use of quinine. Despite the fact that the malarial infections have occupied the attention of the medical profession since the time of Hippocrates, it is not even now impressed with the fact that every such infection is resistant to treatment; that the disappearance of symptoms is no proof that the plasmodia are destroyed; and that a continued course of quinine is absolutely necessary to eradicate even the mildest malarial infection.

The attitude in this respect is very similar to that which obtained in regard to the cure of syphilis prior to the use of the Wassermann test. In both malaria and syphilis specific drugs were known, and if, after the use of these drugs, the symptoms disappeared, the patient was regarded as cured. In the case of syphilis, however, it was recognized that even though symptoms disappeared, from two to three years of constant treatment was necessary before this supposed cure was really accomplished; but in the case of malaria a large proportion of the profession appear to believe that a few doses of quinine, if followed by the disappearance of acute symptoms, signifies the cure of the infection. As a matter of fact, quinine in the treatment of malaria is like mercury in the treatment of syphilis, in that its use must be persisted in for a considerable time after the symptoms of infection have been conquered if one desires to really cure the infection. When this fact is actually realized and acted upon by the medical profession we will witness a great reduction of malaria, because the "carriers" of the disease will be prevented. *The time to begin malarial prophylaxis is with the treatment of every acute infection*, and if this is well done one has performed one of the most important duties, from a prophylactic standpoint, that falls to the lot of the physician. In civil life it may often be impossible to thoroughly treat malarial infections, but in the military service, owing to the absolute control the authorities have over the personnel, the proper treatment of malaria is possible and there can hardly be any excuse for its neglect.

The first requisite to the proper treatment of malarial infection is a correct diagnosis, and, in the military service, this should always be made by the aid of the microscope. This may be impossible in the field and the patient may have to be placed at once upon quinine,

but in camps and posts the microscope should be depended upon in the diagnosis of malarial fevers. In this way the exact species of plasmodium is ascertained and there can be no doubt of the correctness of the diagnosis.

Not only should the diagnosis of malarial infection be made by blood examinations, but the treatment of the disease should always be controlled by frequent microscopic examinations of the blood, and no patient should be allowed to leave the hospital until his blood is free from parasites, except where a military emergency exists justifying such action. If this rule be followed the danger from "carriers" of the infection will be very greatly reduced and the surgeon can not be charged with helping along malarial disease by discharging "carriers" from the hospital, while *gametes* are still in the peripheral blood. If only the forms concerned in the human life cycle of the plasmodia are present in the blood, it will be found that they will disappear within a few days after beginning quinine, and then the patient may be returned to duty with directions regarding further treatment; but if *gametes* are present it will mean from two to three weeks of treatment with the dosage of quinine already recommended before they will be reduced to a noninfectious minimum, and the patient can be safely returned to duty.

The *treatment* of initial and recurrent attacks of malarial fever varies with the species of plasmodium present, the tertian and quartan infections requiring less quinine to cure them than the estivo-autumnal infections. It is not my intention to discuss the treatment of malaria here further than to call attention to the method I have found effective in the usual acute and recurrent infections observed in hospital, the reader being referred to the many excellent works upon this subject for information regarding the treatment of pernicious infections. The object of all treatment is to rid the blood of plasmodia as quickly as possible and to cure the disease, and I am no believer in routine methods in the treatment of infections varying so much in severity as do the malarial fevers. It should be distinctly understood that the methods that follow are merely those that I have found most useful in the vast majority of infections that I have had under personal observation and are inserted merely for the purpose of demonstrating the absolute necessity of continued treatment with quinine and of suggesting the way in which the drug may be employed to secure the end aimed at, i. e., the cure of the infection and the prevention of "gamete carriers."

Tertian and quartan malaria.—In these infections, during the acute symptoms, quinine should be given in doses of at least 0.32 gm. (gr. v) every four hours until from one to two grams are given in the 24 hours (gr. xv to xxx), according to the severity of the symptoms, and

this dosage continued until the symptoms have disappeared and plasmodia can no longer be detected in the peripheral blood. Thereafter the drug should be continued for at least three months, the dosage being gradually reduced during the first two weeks of convalescence until the patient takes 0.40 gm. (gr. vi) per day at the end of the second week, and this dose should be continued for at least two weeks longer, and the same dose given twice a week until three months have elapsed from the date of attack. In regions where reinfection is probable, the prophylactic dose of 0.40 gm. (gr. vi) daily, should be adopted at the end of two weeks after the plasmodia have disappeared and kept up as long as required.

Æstivo-autumnal malaria.—In æstivo-autumnal malaria the dosage of quinine must be somewhat increased, as these infections are more resistant to treatment than tertian and quartan infections. In most cases a dose of 0.32 gm. (gr. v) administered every four hours will result in the disappearance of the symptoms within three or four days, but not infrequently cases will be observed that require larger doses. After the symptoms have disappeared 1 gram (gr. xv) should be taken daily for two weeks, and for two weeks thereafter 0.65 gm. (gr. x) should be the daily dose. At the end of this period 0.32 gm. (gr. vi) should be taken daily for at least two months, and as long as required if quinine prophylaxis is necessary.

Patients should only be returned to duty when the peripheral blood is free from plasmodia, or, if *gametes* are present, when they do not number more than 1 to every 500 leucocytes. Before leaving hospital a malaria register should be prepared for each patient, giving the type of infection, the amount of quinine administered, and other data that may be useful or necessary, and upon this register should be entered further treatment after return to duty. Patients should not be allowed to take quinine away with them for self-administration, but should be instructed to report to the hospital at the proper time and the administration of the drug personally attended to by the medical officer in charge of this work. In case of transfer to another organization, the "malaria register" should be sent to the surgeon of that organization and the treatment continued at the man's new station. In this way every malarial patient treated in the Army can be followed and a really scientific prophylaxis of the disease, so far as he is individually concerned, be rendered possible.

The results of quinine prophylaxis.—In concluding this chapter it may be of interest to give a very few illustrations of the efficacy of the quinine prophylaxis of malaria where other methods have failed or have been only partially successful. Without question the most notable instance of quinine prophylaxis is that which has been in operation in Italy since 1905 under the auspices of the Society for

the Study of Malaria in Italy, led by Celli. In that country quinine is sold by the State to those able to purchase and distributed free to those unable, and Celli claims that since the gratuitous distribution of quinine by the Government the mortality from malaria in Italy has diminished over 75 per cent and the morbidity in proportion. While other methods of prophylaxis, as screening and drainage, have been used, Celli regards the prophylactic use of quinine as altogether the most efficient, and believes that it is the only method that can be relied upon in Italy to control malaria. In view of the great importance of his work I shall give a brief résumé of the results obtained and his commentaries upon them.

In the following table Celli ⁵⁶ gives the results of quinine prophylaxis in the army, in which the morbidity has been reduced from 27.4 per cent to about 5 per cent by the use of this method:

TABLE VIII.—*Malaria in the Army.*

Year.	Number of men.	Number of cases (per cent).	Relapses.	Initial attacks.	Remarks.
1902.....	199,253	27.44	21.41	6.03	No quinine.
1903.....	206,468	24.14	17.85	6.28	Quinine begun.
1904.....	210,637	19.21	12.71	6.50	
1905.....	218,409	21.22	13.04	8.48	
1906.....	211,245	18.69	12.67	6.32	Quinine.
1907.....	202,320	12.46	7.96	4.50	Do.
1908.....	216,679	8.04	5.19	2.85	Do.
1909.....	228,551	6.66	4.72	2.24	Do.
1910.....	234,104	5.10	3.23	1.87	Do.
1911.....	233,517	4.90	3.04	1.86	Do.

From this table it will be noted that there has been a steady reduction in the number of initial attacks and the number of recurrent cases of malaria in the Italian army ever since the adoption of prophylactic quinine, in 1903, until at the end of 1911 the percentage of cases of malaria in the entire army was only 4.9 per cent, and this despite the fact that the troops were often stationed in the most malarious parts of the country.

Celli ⁵⁷ gives the results of quinine prophylaxis in the penal colony of Castiades in the following table:

TABLE IX.—*Malaria in the penal colony of Castiades, Sardinia.*

Year.	Population.	Quinine used, in kilograms.	Number of cases.	Per cent.	Remarks.
1904.....	748	None.	694	92	No quinine.
1905.....	861	13,674	731	84	Little quinine.
1906.....	807	15,070	390	48	Quinine.
1907.....	795	40,230	132	16	Daily quinine distributed.
1908.....	700	35,400	97	13	Do.
1909.....	691	32,000	64	9	Do.
1910.....	655	15,280	139	19	Only curative quinine.
1911.....	540	25,360	37	6	Prophylactic quinine.

In regard to this really remarkable reduction in the number of cases of malaria among the prisoners in this institution from 92 per cent to 6 per cent, Celli says:

It was the prophylactic administration of quinine alone that produced the miracle of diminishing malaria to 8 or 9 per cent.

In the whole of Italy the mortality from malaria has decreased from 21,000 deaths in 1887 to about 3,000 in 1908, quinine prophylaxis having been established about 1905, and Celli says:

Beyond doubt the greatest and most persistent decrease of mortality from malaria in Italy was due to the increased consumption of quinine.

In view of these results it is useless for the opponents of quinine prophylaxis to claim that it is of little value in the prevention of malarial infections, for they absolutely demonstrate that the method, if faithfully followed, is of the very greatest service, and similar results have been obtained by other observers.

In the Dutch Indies, Bisdom⁵⁹ has reduced the percentage of Europeans infected from 80 to 20 per cent, and the percentage of infections in natives from 71 to less than 17 per cent, by the use of quinine in prophylaxis and treatment, and states that the use of this drug has been the most important factor in diminishing the incidence of malaria in the Netherland Indies. In Corsica,⁶⁰ Leger has found quinine prophylaxis very effective, the blood index among the inhabitants of Casabianca, for example, falling from 42.85 per cent to 7.14 per cent after the exhibition of 0.20 gram of quinine daily, and the Sergeants⁶¹ have reported excellent results in Algeria.

The impression that quinine prophylaxis must extend throughout the year in malarial regions is erroneous, for in almost every locality there is a malarial season, and it is only just prior to and during this period of seasonal prevalence that it is necessary to use the drug in this manner. Prophylaxis with quinine should be begun about a month before the malarial season begins and continued for about the same time after it ends. In some localities, of course, the drug will have to be given the greater part of the year, but in most places it will be found that it can be omitted for weeks, or even months, with advantage.

In conclusion, it may be stated that in quinine prophylaxis we possess a most valuable method of preventing malarial infections, and one that can be easily applied in the military service, but it should not be used to the exclusion of the more permanent methods depending upon the destruction of the mosquito, where such methods can be instituted. Although, as Celli says,⁶² "those who take quinine daily, having a certain quantity of it in the circulating blood, can fearlessly subject themselves to the inoculation of blood

loaded with hæmosporidia, and, with much less danger, to the biting of infected anopheles," the method requires the most careful supervision, is more or less disagreeable to the individual, and is less permanent than the abolition of breeding places of the insects transmitting the disease. In the military service its greatest use will be found in the protection of troops in active operations in the field in malarial regions, and as an aid in the prophylaxis of the disease in camps and posts in conjunction with the other prophylactic measures described.

CHAPTER VI.

THE APPLICATION OF THE METHODS OF MALARIA PROPHYLAXIS TO THE MILITARY SERVICE.

In the following chapter a brief summary will be given of the methods of malaria prophylaxis which have been discussed and the manner in which they may be utilized in the prevention of the disease in the military service. The fact that troops are under strict discipline makes it possible to more thoroughly apply prophylactic measures in the Army than is often the case in civil life, and for this reason better results may be expected when such measures are faithfully and intelligently carried out. The great reduction in malaria in the Army already noted is proof of this statement, and there is no reason why these infections should not be still further reduced, and eventually disappear in our permanent posts if prophylactic measures are continued.

As has already been stated, the prophylaxis of malaria requires very different methods in the field than in semipermanent camps and permanent posts, and in all situations the methods employed will vary according to local conditions and military exigencies. This fact must be recognized by every practical sanitarian, and in the following discussion regarding the application of malaria prophylaxis to the military service it should be remembered that the suggestions offered are merely suggestions, which may or may not be practical in actual service owing to the peculiar conditions surrounding each prophylactic problem.

Malaria prophylaxis in the field.—The necessity for malaria prophylaxis in the field during the active operations of an army should have been ascertained before the invasion of the country with which hostilities are being conducted. This presupposes the accurate mapping of the country from a sanitary standpoint, a hitherto much neglected branch of military information. In the past an accurate knowledge of malarial localities would have saved many armies an enormous loss from this cause, as campaigns could have been planned so as to avoid exposing troops to these infections, and the most exhaustive knowledge of terrain is of little use if troops are so decimated by malarial infection as to be unable to take advantage of such knowledge. An accurate sanitary map showing the prevalence of disease in the country to be invaded would be of great value in the planning of any campaign, as in the majority of instances it would be found that badly infected districts could be avoided without dam-

age to the campaign from the military point of view, or prophylactic measures could be instituted in time and prevent the infection of the Army.

In active operations in the field we must depend for the prevention of malaria upon the prophylactic use of quinine and the use of the mosquito net. Quinine sulphate should be given in daily doses of not less than 40 centigrams (gr. vi), preferably administered in 20 centigram doses morning and evening. In many and perhaps the majority of instances it may be found that the entire dose must be given at one time, and if so it is best to give it in the evening. The men should be assembled at a convenient time and the quinine administered by a medical officer, care being taken to see that each soldier swallows his dose. The quinine may be most conveniently carried and dispensed in tablet form, but the utmost care should be taken to see that the tablets are readily soluble.

If acute attacks of malaria develop in the command it will be found best to send the men disabled to the nearest field hospital, where the type of infection can be ascertained and properly treated, or, if necessary, the patient may be sent to the base hospital for treatment. After the plasmodia have disappeared from the blood the men may be returned to duty, but not before. Nothing will be gained by keeping men suffering from acute attacks of malarial fever with an advancing column, as they will only encumber it and may prove a source of infection to their companions.

In all regions in which mosquitoes occur the Vedder mosquito net should be used in the shelter and other tents, and inspections should be made by medical officers in order to see that the net is being properly used. Guards should wear head nets and gloves, and both the mosquito nets and head nets should be inspected frequently for tears or holes and promptly repaired.

It is believed that with an efficient enforcement of the use of nets and of prophylactic quinine an army will be able to campaign in the most badly infected malarial districts without suffering very greatly from the infection. These methods will not prevent all infection, but may certainly be trusted to prevent the vast number of infections which would otherwise occur, and which, in times past, have rendered entire armies useless as fighting machines. If such methods had been efficiently carried out at Santiago it is safe to say that malaria would not have placed our Army in such a position that retreat was actually considered.

Malaria prophylaxis in semipermanent camps.—The first and best step in the prevention of malaria in camps of short duration is the selection of a region free from malaria as the camp site. Here again the value of a map showing such regions is evident, but in its absence, and, if time and military contingencies permit, much may be

learned in a short time by observations of the kind of mosquitoes present, the location of their breeding places, and an examination of the blood of from 20 to 40 children and adults in the region being investigated. This entire study of a location need not consume more than a few hours and will generally result in definite knowledge regarding the prevalence of malarial infection. If, despite the presence of infection, the locality must be selected for a camp site, the measures pursued will vary, of course, with the length of time the camp is to be occupied and the facilities afforded for their execution.

If the camp is to be occupied for only a few days, the prophylactic use of quinine, the use of the mosquito net, and the clearing of an area from 100 to 200 yards around the camp of long grass and underbrush will be about all that can be done in the way of prevention.

In the case of camps that will probably last for several months and are, therefore, of semipermanent character, the same precautions should be taken regarding the selection of a site as recommended for permanent posts, and similar prophylactic measures should be employed if malarial infection appears.

Malaria prophylaxis in permanent posts.—Before establishing a permanent military post in the tropics or in regions in which the presence of malarial infection is suspected, a malaria survey of the locality is of the greatest importance, and, in most instances can be made without interfering with military movements. If such surveys had been made before certain posts were established in the Philippines, it is probable that other localities would have been selected with a resultant saving in money and efficiency. Certainly there can be no excuse for establishing a military post in a very malarious region when a malaria survey would have shown that adjacent regions were free from the infection and when there was no military necessity for occupying exactly the locality chosen.

The following points should be considered in estimating the safety of a locality as regards malaria when the question of choosing it for the site of a post is considered: The statistics regarding fevers in the locality, if there are any, should be studied; the character of the soil and the nature of the terrain; the species of mosquitoes present; the breeding places of mosquitoes and the kind of larvæ present; the presence and number of infected mosquitoes; and the number of latent infections in children and adults. A map of the locality should be obtained, or prepared, and the entire region districted, after which a careful investigation regarding the points mentioned should be made of each district by a trained medical officer. Upon the map should be marked the location of breeding places of mosquitoes, the species found in each breeding place, and whether any of the insects examined were found infected with plasmodia. In addition the dwellings of individuals found infected with malaria during the

blood examinations of the inhabitants should be indicated on the map, thus showing the relation of breeding places to such infections. The map should also show the ratio of infected individuals and mosquitoes and the most severely infected districts. The work of a few days in the examination of the blood of the inhabitants, the dissection and examination of mosquitoes, and the location of breeding places of anophelines will result in a most valuable mass of data, enabling one to judge of the advisability of locating a post in the region investigated, and what methods would be best adapted to ridding the region of malarial infection. Such a malaria survey is just as important to the future health of the command as the question of an adequate and pure water supply.

In posts that are known to be malarial the prophylactic methods adopted must vary with local conditions, and the best way to become acquainted with these conditions is to make a malaria survey, just as one would in deciding upon the location of a site for a permanent post. The post should be districted upon a map and a most careful search made for the breeding places of mosquitoes, while, coincidentally, the species of mosquitoes present should be ascertained and the ratio of infected insects. An examination of the blood of at least 40 adults and children living in the immediate vicinity of the post should be made, as well as of the same number of soldiers in the post, in order to ascertain the presence of latent infection, and the records of the post should be consulted as to the prevalence of malaria during the past. As soon as this preliminary survey is completed one will have data upon which to work out the best method or methods of prophylaxis for the particular locality investigated. While in rare instances it will be found that mosquitoes can be exterminated and that this is the only prophylactic measure that need be adopted, in the vast majority of posts the malarial problem is best attacked by using all the methods at our command. These consist in the abolition of the breeding places of mosquitoes and their destruction in the adult stage by trapping and catching by hand, the protection of the command from their bites by screening and the use of nets, and the destruction of the plasmodia by quinine. The latter method may be omitted, so far as the healthy population is concerned, provided malarial infection is not very intense and the other methods mentioned will control the situation, but in badly infected posts, where it is impossible to get rid of mosquitoes, quinine prophylaxis should be adopted during the malarial season.

Every effort should be made to rid the post and the surrounding country for a distance of at least 2 miles of breeding places of anophelines. Very often the most that can be done is to reduce their number, owing to the immense cost of drainage and filling in, but as

much should be done as possible, however gloomy the outlook. If mosquitoes are very numerous they can be greatly reduced by mosquito traps and by detailing men for the purpose of catching the insects by hand, as is done in the Canal Zone, where this method has met with really remarkable success.

It is unnecessary to state that every post in which anophelines can not be eradicated and where malaria prevails should be thoroughly screened, and yet the cost of this procedure has prohibited its adoption in some of the most malarious posts occupied by the Army, until quite recently. The economy which forced the omission of this very important prophylactic measure was in reality an extravagance, for the loss of time and money occasioned by the malarial infections in the troops was much more costly in the end than the amount involved in the purchase of proper screening material. Barracks, quarters, and other buildings should be screened with the best copper netting of not less than 16 meshes to the linear inch, and, where the yellow-fever mosquito is present, of 18 meshes to the linear inch.

The use of the mosquito net for the bunk should be rigidly enforced unless quarters and barracks are screened, and a nightly inspection should be made to see that the men are using the nets properly. The head net and gloves should be worn by soldiers on duty in the late afternoon and during the night in all posts where malaria is prevalent to any extent.

All shelter for mosquitoes in the post and for a distance of 300 yards outside the post limits should be reduced to the minimum, and the utmost care should be taken that no small breeding places, such as are furnished by unused hoppers, rain barrels, tin cans, and small pools due to improper drainage, exist in the post.

The prophylactic use of quinine should be enforced if malaria is prevalent enough to warrant it, and daily doses of 0.40 centigrams (gr. vi) should be administered under the personal observation of a medical officer. In most posts in which malaria occurs this will not be found essential in the control of malaria, *if "carriers" be discovered and treated and all acute and recurrent cases of malaria be kept in hospital until the plasmodia disappear from the blood under quinine and if treatment be continued in the manner already described.* In the Tropics, however, where the soldiery is continually coming in contact with a badly infected native population, the use of quinine prophylaxis should be insisted upon during the malarial season.

If natives in whom there is a large percentage of latent infection and many "carriers" live around the post, it would be an economical measure for the Government to issue them free quinine, to be distributed and administered by a medical officer. In many instances this measure alone would practically eliminate malaria from among

the troops, because it would remove the greatest source of infection of mosquitoes and, indirectly, of the command.

The discovery and treatment of latent malarial infections and of "carriers" in the command is a most important prophylactic duty, which should never be neglected in posts where the disease is prevalent. This involves, of course, the microscopical examination of the blood of all members of the command, and, in badly infected regions, this examination may have to be repeated at monthly intervals, although the prophylactic use of quinine should render this unnecessary after the first examination has been made. In a large command such an examination may be impracticable, but in smaller posts the measure is practical and, although it involves a great deal of work, should be followed out where malaria is really a serious problem.

Every patient admitted to hospital for malaria should have the diagnosis confirmed by the microscope, if possible, and treatment should be controlled by a daily examination of the blood. No patient should be discharged from the hospital until the peripheral blood is free from plasmodia except in the case of *gamete* carriers, who may be returned to duty when the gametes are reduced to less than 1 to 500 leucocytes.

A "malaria register" should be prepared for every patient, upon which should be entered the type of infection, the treatment given in hospital and ordered after leaving the hospital, the date when the plasmodia disappeared from the blood, the date of return to duty, and any other information that may be thought desirable. When transferred to another command, the malaria register should be sent to the surgeon of the post, who can then continue the treatment.

Every patient returned to duty should be directed to report at a certain time for quinine in continuance of the treatment, and the quinine should be administered under the personal supervision of a medical officer, if possible. The time of reporting can be so arranged as to be convenient for all concerned, and each dose administered should be entered upon the man's "malaria register."

When soldiers suffering from latent malarial infections are discovered they should be placed in hospital and a most careful examination be made relative to the presence of *gametes* in their blood. If *gametes* are not present, they should be returned to duty as soon as the blood is free from plasmodia, with directions to report for further treatment; but if *gametes* are present, the patients should be treated in hospital until the *gametes* are reduced to less than 1 per 500 leucocytes. The method of treating these cases has already been discussed in Chapter V. A "malaria register" should be prepared for these cases and used in the same manner as for acute and recurrent infections, but the fact that the case is a latent one should be entered upon the register.

The suggestions given above for the prophylaxis of malaria in the field, in temporary camps, and in permanent posts, do not cover all that may be done along this line, but may serve as a guide in the prevention of these infections in the military service. The aim of the medical officer should be to do all in his power to prevent the transmission of these infections, but the methods to be adopted will vary, of course, with special conditions, and it will often be possible to control the disease without resorting to all of the methods that have been described. However little he may be able to do in the way of eradicating mosquitoes, he has absolute control over patients in hospital, and can see that no man who has been in the hospital for malaria is returned to duty until he has ceased to be a source of danger to his companions. He can also insist upon the continuance of quinine until there is a reasonable surety that the infection is cured, and thus prevent the recurrences which furnish the majority of malarial cases in any hospital.

In conclusion, it may be stated that the malarial fevers are entirely preventable and their presence, to any extent, in an army post is a reflection either upon the intelligence of the sanitary officer in control, or upon that of other authorities who, either by indifference to the recommendations of the sanitary officer or unwillingness to supply the necessary funds, have rendered the efforts at prophylaxis futile.

REFERENCES.

1. Laveran, A. Note sur un nouveau parasite, etc. Bull. de l'Acad. des sciences. Paris. Ses. 20th Feb., 1880.
2. Ross, R. The rôle of the mosquito in the evolution of the malarial parasite. The Lancet. 1898. II. p. 488.
3. Laveran, A. Ibid.
4. Craig, Chas. F. Classification of the malarial plasmodia. Boston Med. and Surg. Jour. 1909. CLX. p. 677.
5. Theobald, V. V. Monograph culicidæ of the world. 6 vols. 1901 to 1902. British Museum.
6. Howard, L. O.; Dyar, H. G.; Knab, F. The mosquitoes of North and Central America and the West Indies. 1. 1912. Carnegie Institution.
7. Ludlow, C. S. Disease bearing mosquitoes of North and Central America, the West Indies, and the Philippine Islands. Bull. IV. 1913. Surg. Gen. Office, War Dept.
8. Darling, S. T. Studies in relation to malaria. 1910. Dept. San. Isthmian Canal Commission. p. 22.
9. Beyer, G.; Pothier, O. L.; Couret, M.; and Leman. Bionomics, etc. Rep. Mos. Com. to Orleans Parish Med. Soc., New Orleans Med. Jour. 1902. 54. p. 419.
10. Kinoshita, K. Ueber Verbreitung der Anophelen auf Formosa, etc. Arch. f. Schiffs- und Tropenhyg. 1906. X. pp. 621-676, 708-741.
11. Nuttall, G. H. F., and Shipley, A. E. Studies in relation to malaria. II. Jour. Hyg. 1901-1903. 1, p. 45-269; 451, 2, p. 58; 3, p. 166.

12. Stephens, J. W. W., and Christophers, S. R. Malaria in an Indian cantonment. Rep. Malaria Comm. Royal Soc. 1903. p. 22.
13. Ludlow, C. S. Ibid.
14. Hurst. The pupal stage of Culex. Studies from Owens College, Manchester. 1890. 2. p. 47.
15. Chagas. Zeitschr. f. Infectiouskrank Hygiene. 1908. 60. p. 321.
16. Smith. Rep. New Jersey State Agri. Exper. Station. 1904. Trenton. 482 pp.
17. Howard, L. O. Ibid.
18. Craig, Chas. F. The malarial fevers, hæmoglobinuric fever, and the blood protozoa of man. 1909. p. 69.
19. James. Malaria in India. Scient. Mem. Officers Med. and San. Depts. Govt. of India. 1902.
20. Gorgas, W. C. Rep. Dept. Sanitation. Isth. Canal Commission. 1913. p. 46.
21. Le Prince, J. A. Recent progress in antimalaria work, etc. Trans. XV. Internatl. Cong. Hyg. and Demog., Washington. 1913. V. p. 545.
22. Watson, M. Prevention of malaria in the Federated Malay States. p. 952. p. 282.
23. Lutz. Waldmosquitos und Waldmalaria. Centralbl. f. Bakt., 1903, Abt. 33. p. 282.
24. Nuttall. Influence of color upon anopheles. Brit. Med. Jour. 2. 1901. p. 668.
25. Kulagin. Zur Naturgeschichte der Mücken. Zool. Anzeiger. 1907. 31. p. 865.
26. Le Prince, J. A. Mosquito destruction in the Tropics. J. Am. Med. Assoc. 1908. LI. p. 2204.
27. Le Prince, J. A. Ibid. p. 2206.
28. Howard, L. O. An experiment against mosquitoes. Insect life. 1893. V. p. 12, 109.
29. Darling, S. T. A mosquito larvicide, etc. Am. Jour. Public Health. 1912. February.
30. Orenstein, A. J. Mosquito catching in dwellings in the prophylaxis of malaria. Am. Jour. Public Health. 1913. 3. p. 106.
31. Darling, S. T. Studies in relation to malaria. Isth. Canal Com. 1910. p. 32.
32. Craig, Chas. F. The malarial fevers, hæmoglobinuric fever and the blood protozoa of man. 1909. p. 354.
33. Guiteras. Report to Col. J. R. Kean, U. S. Army Med. Corps quoted in Howard's The mosquitoes of North and Central America, etc. 1912. Washington. p. 363.
34. Darling, S. T. Ibid. p. 31.
35. Report of Surgeon General of Army. 1912. p. 147.
36. Howard, L. O. The mosquitoes of North and Central America, etc. Washington. 1912. p. 367.
37. Procaccini. Ricerche profilatt. centri le malaria, etc., Annali. di Medicina Navale. 1900. xi. p. 14.
38. Tzuzuki, J. Malaria und ihre vermittler in Japan. Arch. f. Schiffs u Tropenhyg. 1902. 6. p. 285.
39. Orenstein, A. J. Screening as an antimalaria measure. Proc. Canal Zone Med. Assoc. 1912. v. Part I. p. 12.
40. Craig, Chas. F. Studies in the morphology of the malarial plasmodia after the administration of quinine, etc. Jour. Infec. Dis. 1910. 7. p. 285.

41. Rieux, J. Mode d'Action de la quinine sur *Plasmodium vivax*, etc. Bull. Soc. Path. Exot. 1913. 6. p. 153.
42. Billet, A. Action de la quinine sur les hæmatozoaires de paludisme. Bull. Soc. Path. Exot. 1913. 6. p. 336.
43. James, W. A. Personal communication.
44. MacGilchrist, A. C. Pharmacological action and uses of quinine. Proceed. Third Meeting Gen. Malaria Committee. Nov. 18. p. 17. Simla. Gov't Press.
45. Koch, R. Zweiter bericht uber die thatigken des malariaexpeditio. Deutsch. Med. Wochenschr. 1900. 5. p. 88.
46. Thomas, H. W. The sanitary conditions and diseases in Manaos, etc. Ann. Trop. Med. and Hyg. 1910. iv. p. 7.
47. James. Scientific mem. officers med, and san. depts. Govt. India. 1902. N. S. No. 2.
48. Craig, Chas. F. Observations upon malaria. Latent infections in natives of Philippine Islands. Phil. Jour. Sci. 1906. I. p. 523.
49. Ollwig, R. Zeitschr. f. hygiene u infectionskrank. 1903. 45. p. 345.
50. Sorel, F. Le paludisme a la cote d'ivoire. Bull. d. la Soc. Path. Exot. 1912. x. p. 365.
51. Plehn, A. Die acuten infectionskrankheiten bei den negern, etc. Virchow's Archiv. 1903. Supplement.
52. Sergent, Edmund and Etienne. Atti. della soc. per gli studi della malaria. 1909. x. p. 217.
53. Darling, S. T. Ibid.
54. Darling, S. T. Ibid.
55. Thomson, D. The destruction of crescents. Ann. Trop. Med. and Parasit. 1912. 6. p. 223.
56. Celli, A. The restriction of malaria in Italy. Trans. XV Internatl. Cong. Hyg. and Demog. 1913. v. p. 526.
57. Idem. Ibid. p. 528.
58. Idem. Ibid. p. 525.
59. Bisdom, W. Einige bemerkungen uber die malaria im indischen heer in den Jahren, 1895-1909. Janus. 1912. 17. p. 400.
60. Leger, M. Le paludisme en corse. Pub. de l'Inst. Pasteur. 1913. Laval.
61. Sergent, Edmond and Etienne. Etudes epidemiologiques et prophylactiques du paludisme. Ann. Inst. Pasteur. 1913. 27. p. 373.
62. Celli, A. The restriction of malaria in Italy. Trans. XV Inter. Cong. Hyg. and Demog. 1913. v. p. 522.

MONOGROPHS UPON THE MALARIAL FEVERS.

1895. Thayer and Hewetson. The malarial fevers of Baltimore.
1897. Thayer, W. S. Lectures upon the malarial fevers. New York.
1900. Marchiafava and Bignami. Malaria. Twentieth century practice. New York. Vol. XIX.
1901. Craig, Chas. F. The aestivo-autumnal malarial fevers. New York.
1904. Stephens and Christophers. Practical study of malaria. London.
1905. Ziemann, H. Malaria. Handbuch der tropenkrankheiten. Mense. Leipzig.
1909. Craig, Chas. F. The malarial fevers, hæmoglobinuric fever, and the blood protozoa of man. New York.
1913. Ruge, R. Malariaparasiten. Handbuch der pathogenen microorganismen. 2nd auflage. BDVII.



